


 The logo for the journal Nature, featuring the word "nature" in a white, lowercase, serif font inside a dark rectangular box.

February 5, 1976

Select Committee needs a better analysis than this

It is depressing that the House of Commons Select Committee on Science and Technology, in its latest report on research in universities (HC87; HMSO, 75p), should have marred an on-the-whole sensible and careful document by some ill-considered remarks about "a handful of highly favoured scientists" who "may influence the formulation of Science Research Council (SRC) policy". Even though the committee protests that the paper is an interim one, and that it is only asking questions and seeking out topics for further investigation, there is cause for concern that it should have gone into print with an appendix in support of this musing which can by no stretch of the imagination be called well researched.

This appendix argues that a few scientists get a lot of money. The list of 3,000 SRC grants in operation in 1973-74, totalling £53 million, was analysed, and it turns out that the top 2% of recipients get 20% of the funds (in grants of £100,000 or greater), and 10% get 45% of the funds. The mean value of grants issued by the Nuclear Physics Board (which also comprises high energy physics) is ten times that of the Mathematics Board. And people who accumulate more than one grant are more likely to be those who are already in receipt of large grants. (This rather depends on whether you believe that the 13 out of 31 recipients of big grants who receive further grants is a significantly higher fraction than the 307 out of 919 recipients of relatively small grants who land a second grant.)

The report's analysis of the trend with time shows that in 1974 almost no grants were awarded for a period of longer than three years; in earlier years 20% fell into that category. Finally, the mean grant size in nuclear and space science seems to have leapt up very significantly in 1974.

The conclusion is that there is a "concentration of large resources in a handful of outstanding individuals" which "seems to indicate at the very least that SRC policy is as much influenced by the demands of a small core of scientists as from any centralised decisions about what lines of research ought to be pursued". Nuclear physics takes a "disproportionately high percentage" of the budget; the SRC does not seem to be making any long term investments, and the increase in nuclear and space grants "would seem to indicate an increased commitment to expensive projects".

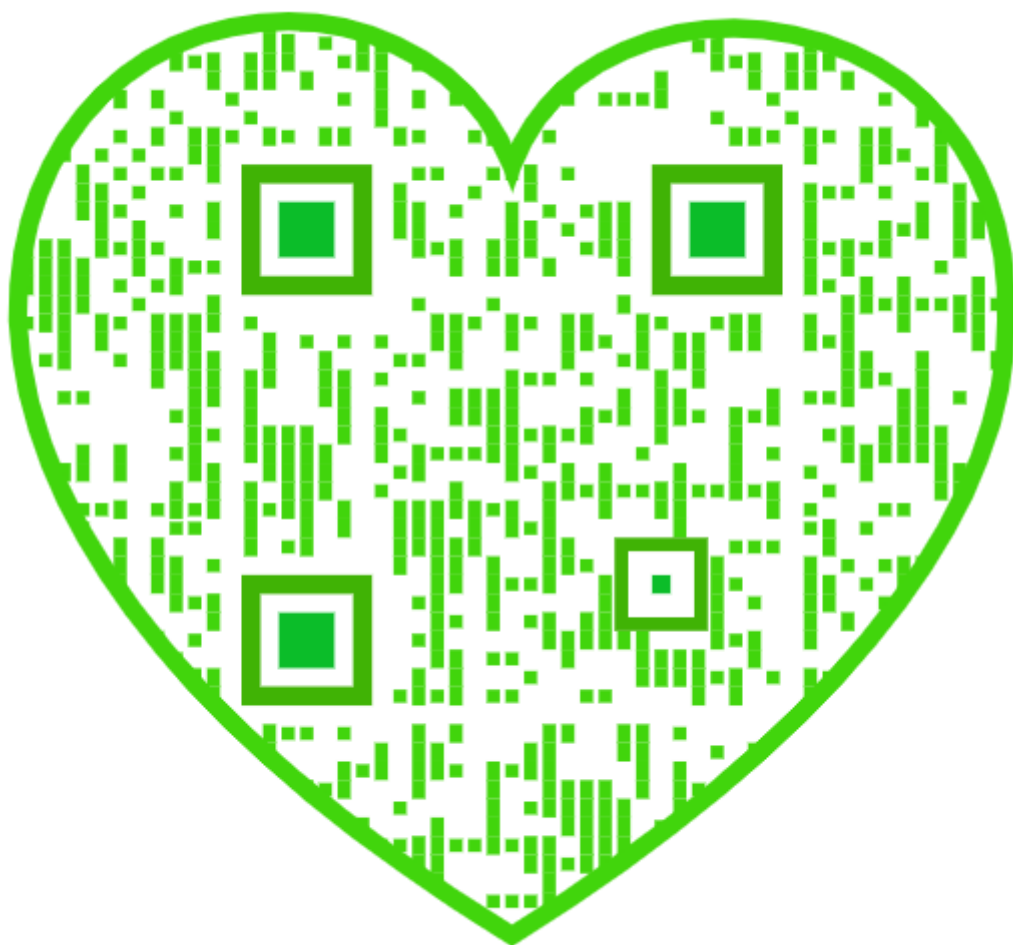
The evidence on which these conclusions or indications are based is so incomplete and selected with such ignorance of how science funding operates that the select committee should disown the report. It represents the worst sort of sociology of science in which actually talking to people is regarded as a poor substitute for computer analysis of a mass of data. Had the compiler of the report spoken to the SRC (which he or she palpably did not) before attempting to guess motives and policies, a much more profound analysis could have been made. As it is, the most glaring nonsense is talked of highly favoured individuals, "36

researchers control 23% of the budget" and so on.

Two things need to be said about this apparently charmed circle of researchers. First, some science is necessarily very expensive and requires major capital investment. To deny such expenditure simply because it is widely different in scale from that for most scientific endeavour is to condemn British science to small-minded egalitarianism. Second, the circle is not small at all. Had the committee investigated who gets the big money, they would have found that there is no room on the SRC grant application form to list co-investigators. In some cases the head of a department or a large facility (such as a radiotelescope) secures all the money under one heading, for administrative convenience, and may have 30 or more staff on the grant, pursuing independent work. In other departments, individuals go for their own grants. Thus talk of funds being concentrated among relatively few researchers is meaningless if the committee's investigations are carried out so superficially as to consign this central issue to an observation in a footnote that "research funds probably reach a wider population".

The analysis of temporal trends is equally badly under-researched. Remarks are made about the SRC's "increased commitment" to expensive projects, on the basis of a growth in the grants to space and nuclear science. This "growth" is based on a comparison between grants awarded before 1974 and still current in that year, and those awarded in 1974. But this is no comparison at all without knowledge of funding policy. If the grant committee awards money for relatively cheap research posts (assistantships and fellowships) on a three-year basis, and large capital expenditure on a one-year basis, every new year will see a carry-over from past years of relatively small sums in continuing grants, but the award of large sums for the one year. The average grant continuing into 1974 in nuclear physics was £428 a month. The average new grant was £4,359 a month. Nine hundred per cent growth or an unimportant indicator of detailed grant-giving policy? The report gives no indication that anyone tried to find out. Clearly no-one read the SRC's last annual report on the question of financial strategy. It is deplorable that such a slipshod job coupled with pure guesswork should pass for an analysis of the SRC's policy.

Science is increasingly suffering from divisiveness these days, as different groups are set against each other. If in the name of egalitarianism this fatally flawed report is accepted and used as the basis for recommendations, the committee will have to bear the responsibility for the further decline in morale among those in big science. And the clear bias against nuclear and space science pervading the report looks more than a little ridiculous when it turns out that 60% of those big grants actually go to fund engineering projects. □



Before Italy's latest government crisis, the then Minister for Research, Sr Mario Pedini, forecast that 1976 would be the turning point for Italian science. **Gillian Boucher** reports.

Trying to make Italian scientific research useful

TO ACHIEVE one's own ministry must be a cherished hope of many a minister without portfolio. Italy's Minister for the Coordination of Scientific and Technological Research has little real power—he does not, for example, appoint the President of the CNR (National Research Council). But Sr Mario Pedini's prophecy rested on the progress of a bill being examined in Parliament for the creation of a Ministry for Scientific Research. And he spoke for many Italians when he argued for more surveillance over research. Along with the general despair over the way that both education and industry are run there has recently arisen the strong feeling that the solution lies in science—that if only academic pursuits could be programmed to the needs of the country all would be well.

In a brief history of Italian research written for a permanent commission of the Senate, A. Alberigi Quaranta summarises the attitudes which led to the current state of affairs. Italian science, stifled by the Fascists, got off to a bad start after the war. American generosity was largely to blame for this: it disguised the need for industrial innovation and provided the means for many of the brightest young researchers to emigrate. In the 1960s, while pure research was flourishing, at least in patches, the state of applied research remained pitiful. This, the argument goes, stemmed partly from the strong inclination of Italian education and culture towards theory as opposed to application, partly from the attitudes of industry, in particular of the many small firms which had not the resources for research or development, and partly from the lack of guidance on the part of the government.

Against all this there is now a violent reaction. Most of the Ministers of Research of the last ten years have in fact supported some project or other for a ministry. But the issue is now a much hotter one. Parliament has been examining two proposals for a ministry: Sr Pedini's bill, recently approved by the late government, and an extra-governmental one supported by parliamentary Christian Democrats, Communists, Socialists and Liberals.

The latter parliamentary text sees the function of the ministry as co-ordination and political direction of research: these powers, it says, would be withdrawn from the CNR. It pro-



Mario Pedini: prophecy

poses that a National Science Council should be created within the compass of the ministry, and that there should be both a national plan for research and a national fund for implementing it.

The chief difference between this proposal and the government bill lies in the government bill's proposal that the ministry, in addition, should administer research. Public bodies, and any private firm which wants public money for research, would have to present their research programmes to the ministry. The ministry would promote certain research by creating some ill-defined 'research centres'. University research would be coordinated and directed by means of a yearly conference, the composition of which may be decided by the Prime Minister. The CNR would give technical advice to the ministry and would execute its plans. Its president would be appointed by the Prime Minister, on the advice of the Minister for Research; at present the President of the CNR is appointed by the President of the Republic.

An exceedingly optimistic timetable is laid down for the yearly funding of science. Publication of the report of the conference on university research is set for this month. All research proposals will be submitted by March 1 to the ministry, which will judge them according to whether they fulfil a need recognised by the government, and whether they will duplicate

other research. The Minister will report at the end of April on the state of research and the value of the submitted projects, and the Interministerial Committee for Economic Planning (CIPE) will make its final report to the Treasury by May 31.

Reactions to the proposals in academic circles have been mixed. On the one hand many academics want to do something useful for their country, and they welcome the possibility of an end to their frustration at the lack of contact between universities and the outside world. On the other hand there is the fear, far from unjustified in Italy, that anything the government touches will grind to a halt, stalled by bureaucracy. The new enthusiasm for coordinating everything is also worrying. The universities are hardly mentioned in Pedini's bill. The sums for research they receive at present from the Ministry of Education are negligible. Pure research will clearly be in a very unhappy position if its only source of funds is through a ministry whose purpose is to administer science "in relation to the technical, social and economic progress of the country". Answering a query about his intentions for universities, Pedini has said, "Let's start with the ministry, and when it has reached puberty we'll mate them"—which is hardly reassuring.

Certain obvious questions beg themselves. Why a ministry, when a body already exists which funds and co-ordinates science, namely the CNR? Why could the CNR not forge the links between research and development, between university and industry? Part of the answer is that the CNR has tried, and failed. A total of 28,000 million lire were spent between 1964 and 1972 on twenty applied research projects; the CNR's own judgment was that most of these were unsatisfactory. Though the scientific merit of the projects was considered carefully when they were planned, there was no proper investigation of whether development would be economically feasible, or even whether there was any Italian industry in a position to use the results. The CNR, in fact, is not equipped to make decisions about economics and industry: it represents the interests of academics, with 128 of the 140 members of its Scientific Committees academics, and members of these committees also on its Administrative Board.

But the CNR, undeterred by these drawbacks and alarmed by the prospect of a ministry, is having another go at applied research. In 1972, after consulting researchers and the Office for Economic Development, it put forward 78 proposals for research projects with potential economic or social applications. By the beginning of 1975 an initial selection had reduced the number of projects to about 40, grouped into seven themes: energy (eg. solar and geothermal energy, conservation of energy); food (eg. increasing plant productivity, new sources of protein, mechanisation of agriculture, fisheries research); health (eg. preventative medicine, viruses, reproduction, biomedical technology); environment (eg. geodynamics, oceanography, soil conservation); educational theory; advanced technology (eg. telecommunications with centimetre and millimetre waves, power lasers, superconductivity, ceramics, control of air traffic); and cognitive advancement (a ragbag including criminology and astrophysics).

These projects, together with assessments of their feasibility and usefulness, were submitted to the CIPE in April 1975. The CIPE's ruling, published on October 9, was startling. The entire 'Health' programme and almost all of 'Food' were approved. 'Energy' and 'Environment' were heavily lopped; nothing remained, for example, of the projects on solar and geothermal energy. 'Cognitive Advancement' and 'Educational Theory' were rejected, at

least for the present. But most surprising, after all the trumpeting about the economic needs of the country, 'Advanced Technology' was almost completely rejected: only the project on the control of air traffic remains.

It is possible that the government has simply lost faith in the CNR's ability to know what industry needs, and prefers to wait for the Ministry for Research. This might be deduced from the extremely fuzzy explanations the CIPE report gives for its decisions. Perhaps the CIPE is concerned to prevent toes from being trodden on—the National Electricity Board (ENEL), for example, may consider research into new sources of energy its own preserve. It may also be considering the violent public dislike of multinationals, which could see the research on telecommunications, for example, as somehow pandering to the Americans.

In any case about 20,000 million lire have been set aside for the first year's research on those projects which have survived scrutiny. Owing to administrative delays it will not actually be handed out until towards the end of this year. The CNR's 1975 grant of 83,000 million lire is increased this year to 100,000 million lire, which means 3,000 million lire less for other research of the CNR—a blow at any time but especially with inflation.

Will the success of the applied projects make the loss worthwhile? Unfortunately the answer is likely to

be no. The projects suffer from the defect seen in all CNR funding, which is ultimately the fault of the way Italian universities are organised. Substantial amounts of money are divided into too many little sums, with the result that there is much duplication and nobody has the resources for a major piece of work. The practice arises because Italian universities are divided into small faculties, usually with one full professor each, which jealously maintain the barriers between themselves and other faculties. The CNR has naturally never challenged this arrangement, and nothing has changed with the new applied projects: only one quarter of the money is going to CNR institutions, some at least of which are big enough for a more flexible, interdisciplinary approach; most of the rest is destined for hundreds of university faculties.

Given this, and the fact that little thought seems to have been directed at precisely how the results achieved are to be made available to those who might use them, the projects seem likely to cause little more than a ripple in Italian economic or social life. But one thing to be thankful for is that the money is there to do something with. If a large part of it ends up financing the pure research that the academics really want to do, many would say so much the better. But when the Ministry for Research is created, money for pure research may be difficult to come by. □

CANADA

Getting the message across

David Spurgeon reports from Ottawa on science and the mass media in Canada

A STUDY on communication of science through the mass media confirms what Canadian scientists, science writers and ordinary readers have known for a long time: that the Canadian media do an inadequate job of making science meaningful to their public. But, surprisingly to some, it also indicates that a far greater public desire exists for science coverage than most media managers seem to realise.

The study, begun in 1973, was published recently by the Canadian

Ministry of State for Science and Technology (MOSST)*. It indicates, for example, that science writers are willing and anxious to do a better job, but that they are hindered by the out-dated ideas and prejudices of their bosses—those who control what the media transmit to the public. At the same time, it reveals that much of the general public is just as interested in learning about science as about those other human activities that receive preferential treatment by the media; yet because of the attitudes of the media managers, this desire goes unsatisfied. The study shows how great the need is for better science coverage, for it reveals the abysmal ignorance about Canadian science on the part of those



Photo: TTC

Toronto Transit commuters: does the Canadian public at large get the science coverage it wants?

same members of the public who want to know more.

The results contained in the second volume, released to the public last month, are the outcome of three separate surveys made of managing

**Media Impact, Volume 2, Science, Mass Media and the Public, A Research Study on Science Communication.* By Orest Dubas and Lisa Martel (Information Canada; June 1975).

editors of daily newspapers with a circulation of more than 5,000, science writers and the public at large. Volume 1 contained an outline of objectives and methodology and gave an account of past research on science communication in other countries.

The public survey was conducted between March and April 1974 by the private firm, Canadian Facts: 2,000 Canadians, aged 15 and over, were randomly selected for interview. The surveys of managing editors and science writers were conducted by the authors through questionnaires. Editors of 52 of the 81 newspapers polled responded. From 176 writers (only 24 of them employed to write science or medicine full-time for daily newspapers) 113 replies were received.

Some indication of the general level of awareness of science affairs in Canada can be gained from the finding that two out of three people interviewed could not even name a single scientist. Of those who did recall one or more names, less than one in five could name more than two. And when names were mentioned, they were mostly the expected ones: Frederick Banting and Charles Best, co-discoverers of insulin, were mentioned about half the time, and—American claims for the invention of the telephone to the contrary—Alexander Graham Bell was mentioned by another third. Only seven persons among the 2,000 polled could recall the name of Dr Gerhard Herzberg, the physicist who won Canada's only Nobel Prize in the natural sciences almost half a century after Banting. Best and MacLeod were awarded the country's only Nobel Prize in medicine in 1923.

To make things worse, some of those interviewed tended to confuse Canadian scientists with those of other countries, or even with public figures who were not scientists at all. For example, 7% cited names like Albert Einstein, Louis Pasteur, Eve Curie, Charles Darwin, Jonas Salk and Alexander Fleming when asked for names of Canadian scientists. And occasionally they brought up names like Lester Pearson (former prime minister), Mme Jeanne Sauvé (former science minister), Farley Mowat (a writer on nature themes), and Fernand Seguin (a French Canadian science communicator).

When it came to Canadian scientific achievements, 61% failed to name one, 22.8% listed a single achievement, 9.7% mentioned two and 8.1% three or more. The discovery of insulin and the invention of the telephone were remembered as Canada's greatest scientific achievements, and most of the optional "projects" mentioned were really technological or engineering feats, such as the St. Lawrence Seaway or the Mirabel airport. Fewer than a

dozen mentioned the CANDU nuclear power reactor, one of the most successful designs in the world. Many of those who recalled a Canadian scientific achievement did so because they were personally involved in it, or knew somebody who was. Thus insulin, for example, was remembered because a respondent "knew the first people treated", and a heart machine because they "used it on me at the hospital."

Significantly for the purposes of the study, the mass media appear to be the major source of what little public awareness there is of Canadian science. Nearly half the respondents cited the media as their major source of information. Magazines were the main source for science news, and were preferred over newspapers and television. Radio ran a poor third, which was surprising because the State-subsidised Canadian Broadcasting Corporation provides many good science programmes; more than half the respondents, however, said they were not aware of any of the science or public affairs programmes mentioned to them. Despite their evident ignorance of the subject, 82% felt it was important to be kept informed about science, 63% were interested in finding out more about Canadian achievements, and more respondents than not (42.8 to 39.6%) thought the media were providing an adequate coverage of science.

Furthermore, without being told that the interview focused on science, the public sample rated news of science and medicine high compared with news about such other things as sport, entertainment, society, politics and foreign affairs. Medicine and health ranked third in interest after news and education, and pollution and biology rated higher than entertainment. More indicated an interest in research in the physical sciences than in sports. Crime was third last on the list of 23 items of interest, following only after a number of scientific categories. And the public even declared itself willing to give up some coverage of some of the non-scientific categories in order to learn more about science.

To anyone who has written for the public about science for years, in fact, many of the public's responses as indicated by the survey were hard to believe. One obvious weakness of the survey was the breadth of its definition of science: it included not only the natural sciences, life sciences and engineering, but also the social sciences and even the humanities, under which such topics as education, business and economics were listed. And one apparent anomaly was the finding that less than half the interested readers judged science articles in newspapers to be accurately reported. In view of their

obvious ignorance of the subject, how would they know? Three out of five, on the other hand, believed magazine articles (at least those about life sciences) to be well reported, and two out of three felt television reporting was accurate—in fact, television was perceived as the most accurate of the media, despite the fact that few magazines or television stations in Canada employ science specialists.

The Communicators

The study's survey showed that the science writers—the people initially responsible for communicating news of science—themselves believe that the media cover science inadequately, both in quantity and quality. They, too, judged the poorest medium to be radio (which, outside of the CBC, also employs few science specialists). They felt that one of the most important obstacles they faced in reporting science was the need to cover such a wide range of topics (more than one in three wrote on at least 10 fields of science a year), but they also pointed to the difficulty in keeping stories at once simple and accurate, and the lack of time they had to research their stories. The biggest external obstacle was the traditional reluctance of scientists to communicate with the public.

A profile of the science writers revealed that 69% had a university degree, but that twice as many had training in writing and the liberal arts as in the sciences. About 40% took either supplementary training or university courses in the sciences.

The managing editors survey showed less enthusiasm for science coverage and more complacency about the way it was currently being covered than either the public or the science writers. As the authors of the study summarised it: "Editors' replies (to a section asking if they anticipated changes) were indicative of management views on most papers: that all was well with science coverage." While nearly half the papers surveyed had assigned reporters to cover medicine and health, only 22% (11 papers) had assigned a staff member to cover science. Five of these science reporters were on papers with circulations of more than 75,000. A variety of reasons were offered for not having a full-time science writer. Many editors said they could not afford it or found it cheaper to get the news from the wire services. And 29 of 33 editors who replied said their papers did not have enough staff-written science news to justify a full-time science writer—a neatly circular argument, as one critic put it.

Although both the public and the science writers who were surveyed thought it was a good idea to set up science news in a regular, easily-identi-

fied way, the editors were less enthusiastic. Between 40 and 49% of the public agreed that, when they are specifically looking for science articles in the press, they have difficulty finding them. About 68% of the science writers would like to see a weekly column on science in the press, and 44% preferred a full page weekly devoted to science. Yet among the editors, only 31% believed readers would like to see some sort of regular science feature; 70% felt that science should be presented only as items became available, and only five of 52 editors thought readers would want a full page weekly.

Perhaps some clue can be found in the educational backgrounds of the editors: less than one in three had any science courses at the post-secondary school level, and only 31% had a university degree (compared with 69% of the science writers). Although 20% of the editors reported they had had science or technical writing experience themselves, it ranged from one whose experience was "sporadic" to three with more than 20 years' experience.

The authors of the study draw the following conclusions:

"There is as yet a dilettante, hit-and-miss approach to science coverage in general, and Canadian science in particular. Even the barest informative functions of the press are not met in this area, let alone the interpretive or educative role (which is becoming more important as science affects the readers more and more).

"Often only the highlights of a scientific meeting or the outline of a project—with little relevance to impact on society are presented; in many cases by news services reporters and not by staff writers of the paper... Finally, even if good stories are written, they can still get postponed or not even used—frequently superseded by the most trivial, sensationalized items; or else buried in the paper where only the hardy will ever find them."

However, like good journalists, the authors did at least give something of the other side of the argument. One of the editors quoted, from a paper of approximately 50,000 circulation in the prairie provinces, commented: "The whole tenor of the questionnaire is so unrelated to newsroom practice that answers cannot have any validity. For example, anyone knows that any particular story has 100 facets, and to attempt to departmentalize 'science' in the modern world is meaningless. And while on any one day, no one may edit what you call a 'science' story, the next day 10 people may deal with 15 so-called 'science' stories. Go into the newsrooms and you'll find your answers. First of all, define 'science' news." □

BRITISH STEEL

Quiet change at BSC

ALTHOUGH the spearhead of the British Steel Corporation's attempts to stem its losses and revitalise itself depend to a great extent on agreement with the trade unions to a reduction in the total labour force (some 220,000 people at present), a step forward in rationalising its research and development was taken recently with the minimum of fanfare and publicity. Quite simply, the BSC now has a Chief Scientist and a Controller of R and D, in the true Rothschild tradition of the "customer-contractor principle".

The annual research budget of some £13 million may not seem much by comparison with the corporation's losses, which are now running at about £8 million a week, but when the BSC is properly on its feet again, it will undoubtedly profit from the more razor-edged research arrangements that now seem in prospect.

As from the beginning of last December, Dr Robert S. Barnes, until then Director, Research and Development, became Chief Scientist, and Mr James Mackenzie, who had been Technical Director of the General Steels Division, became Director, Research Laboratories. This means, essentially, that Dr Barnes says what he wants done and Mr Mackenzie establishes that it can be done and sees that the work is carried out.

At present the two men are sizing their new jobs up and it will be a few months before they finally sort out precisely how their responsibilities should be divided. It is fairly certain, for example, that Dr Barnes will have under him a team which will be responsible for investigations into the cost-benefit of research done by the BSC and for techno-economic analysis. Until December, research was carried on within the corporation's four divisions and at three "corporate laboratories" which used to make up the old British Iron and Steel Research Association, BISRA. This somewhat decentralised system has its roots back in the days before British Steel was nationalised, and it is no secret that there has been some overlap and duplication of work, which admittedly has been decreasing over the years.

The corporation has no plans to close any of the laboratories or to cut its spending on research, and any redundancies that may occur will be part and parcel of the BSC's overall labour strategy. The changes do mean, however, that laboratories will lose some of their autonomy under Mr Mackenzie, who will be based in Teesside in North-east England. And there

seems little chance of major new research avenues being funded with new money in view of the corporation's perilous financial situation.

These changes in research and development do little, of course, to help the corporation with its immediate problem of turning itself into a viable entity. Since nationalisation in 1967, BSC's capacity of some 27 million tonnes a year has been maintained while the number of individual steel plants has been reduced from 40 to 30, and the corporation's basic aim is to produce some 80% of a projected output of 35–37 million tonnes (in the mid-1980s) in just five major integrated steel complexes—two in Wales producing 9.5 million tonnes a year between them, two in North-east England with a combined output of 16 million tonnes and a Scottish complex (4 million tonnes). In these giant plants, ore will be turned into a finished product on a single site. The remaining 20% of the output will be mainly of special steels, and this activity will, as at present, be centred on Sheffield.

Up-to-the-minute though this may sound, BSC will be doing no more than the Japanese and others have already achieved: the Nippon Steel plant at Fukayama, for example, has an annual output of some 16 million tonnes, and that at Dunkirk in France produces about 8 million tonnes a year. It is widely agreed that a really viable steel plant must be sited near a deep-water terminal (thus eliminating the relatively very high cost of transporting ore by rail) and should have an output of several million tonnes a year (thus opening up the advantages of ore transportation in 250,000-ton bulk carriers).

Roger Woodham



Photo: Harrison & Laking

Robert Barnes, Chief Scientist at BSC.

US ENERGY

Saving without changing

An aggressive energy conservation programme would enable the United States to meet all its energy requirements for the next 25 years without recourse to environmentally destructive new sources of supply, according to a provocative study published this week. Colin Norman reports from Washington.

FULLY half of the energy now used in the United States is wasted, and massive savings are possible without major changes in the American way of life, according to a study written by Denis Hayes, a former energy adviser to the Governor of Illinois. The study was published by the Worldwatch Institute, a relatively new Washington-based research organisation whose previous analyses of global problems, such as food supply, have attracted considerable public attention.

The conclusions reached by Hayes run counter to the conventional wisdom that energy consumption is so closely correlated with standard of living that major reductions in energy use would precipitate severe economic dislocations and cause living standards to drop drastically. Hayes says, however, that his conclusions rest on the assumption "that lifestyles will change only cosmetically—that Americans will continue to travel as many miles, keep their homes just as warm, operate as many appliances, and eat what they now eat". Moreover, he argues that a strong energy conservation program would actually increase employment and "save consumers billions of dollars a year".

Hayes told a group of reporters last week that he reached his conclusions about energy conservation after becoming convinced of the potential difficulties in opening up new sources of supply. Noting that economic and technical problems have caused some of the leading industrial contenders to drop out of the effort to develop oil shale, that increased use of coal would create unacceptable environmental pollution, and that nuclear energy is beset with troubles, he said that "source after source is becoming increasingly less optimistic". Moreover, since oil and gas reserves in the United States are dwindling fast, Hayes suggested that "we are consuming energy like a childless society", rapidly depleting energy reserves "with little regard for the future energy needs of our children".

The American way of life is "rife with opportunities to conserve energy", Hayes says in his report.

- The major opportunities lie in transportation. Hayes estimates that some 42% of total energy consumption in the United States is accounted for, directly and indirectly, by transportation; he reckons that half of that amount could ultimately be saved. His suggestions include gradually tripling the mileage performance of vehicles, mostly by reducing the weight of automobiles; greater use of public transportation and car pooling; and shifting freight from trucks to more efficient means of transport such as railroads and waterways.

- Stricter insulation standards for new buildings and increased insulation of existing structures, coupled with greater use of solar energy for heating and air conditioning, could save as much as 16% of present consumption, he calculates.

- Technical improvements in agricultural production and changes in the way food is packaged and sold could save another 5%.

- Improvements in the efficiency of electricity generation, through more use of waste heat and better generating technology, could save 3% of present energy consumption.

- Finally, greater use of recycling and elimination of unnecessary packaging could save 4% of current energy needs.

Few of those suggestions are new. In fact, most of them were aired in a massive study of energy policy options for the United States, published in 1974 by the Ford Foundation's Energy

Policy Project. The catch, of course, is that the road between devising an energy conservation programme and implementing it is paved with political problems. Though Hayes notes in his study that such problems exist, he offers few suggestions for overcoming them.

To achieve the level of energy saving which his study suggests would require considerably increased government regulation of various industries, use of tax incentives for increasing efficiency and other similar devices. Most of those measures would be opposed by a powerful coalition of industrialists, energy interests and financiers.

But perhaps the chief political problem is that the greatest single incentive for energy conservation would come from greatly increased energy prices, which are still comparatively low in the United States. Though he does not discuss prices at length in his study, Hayes said last week that he would personally favour much higher prices than even a free market would produce (natural gas and oil prices are now controlled to some extent by the federal government). But he added that if prices are allowed to rise, an income distribution scheme would be required to prevent the burden falling on the poor. That, of course is a politically explosive issue. Last year, Congress balked at decontrolling oil and gas prices, partly because of the suspicion that oil companies would be able to reap greater profits if controls were removed, but mostly because of the fears of a voter backlash provoked by rising energy costs.

Boost for biomedical research

CONGRESS last week handed President Ford a stunning political defeat when it voted, by a surprisingly large margin, to ram a budget bill for the Department of Health, Education and Welfare (HEW) into law over his objections. By its action, Congress has lifted the sagging budgetary fortunes of the National Institutes of Health, for the bill contains a large helping of money for biomedical research—considerably more than Mr Ford wants to spend. There are still a few political battles to be fought before NIH can spend the money, however, and the exact amount and the timing of its release by the Treasury are not yet certain.

The HEW budget bill—which applies to the fiscal year which began more than 7 months ago—has been tangled in a struggle between Congress and the Ford Administration over spending priorities. The supporters of the overriding action have

argued that since Congress had already cut the defence budget, an increase in the HEW budget would not add to total government spending, but merely shift priorities from defence to domestic programmes.

As far as NIH is concerned, Mr Ford had proposed that it should receive slightly less money this year than it received in the 1975 fiscal year. The bill passed by Congress, however, will increase NIH's budget by some \$200 million.

Mr Ford might still be able to delay the expenditures and perhaps, reduce them a little. In the next few days he will formally propose to Congress that some of the money should not be spent this fiscal year; Congress will have 45 days in which to act on that proposal. That means NIH will not get its budget sorted out until three quarters of the fiscal year has gone by. Another battle will then begin over HEW'S Fiscal year 1977 budget.

BRAZIL

Two Brazilian ecologists are making progress in their fight to save the "mico"—a small, round-faced, long-tailed monkey of the genus *Cebus*—from extinction. A generation ago, when Rio de Janeiro's famous Copacabana beachfront neighbourhood was a quiet, distant retreat for fishermen and weekend trippers, with hardly any permanent residents, countless thousands of micos played in the branches of trees that proliferated in that district. Now, with Copacabana turned into an overpopulated jungle of high-rise apartment buildings, almost no micos are left in Rio. (Fewer than 1,000 are known to exist in the whole of Brazil.)

For several years, however, ecologists Adelmar Coimbra Filho and Alceu Magnanini of the Rio State Engineering and Environment Foundation, have been battling to prevent the mico from disappearing. They started out by keeping a few micos in the safety of their back yards and encouraging them to breed. In 1972 they obtained a foreign grant and created a biological preserve in Rio's still-untouched Tijuca Forest. Today the mico population there has grown to 58.

The prospects for keeping the mico from becoming extinct are now better than ever before. Brazil's federal Land Reform and Colonisation Institute has promised Coimbra Filho and Magnanini that it will expropriate a wooded tract of land north-east of Rio—a former natural habitat of the mico—and turn it into a restricted-access biological area where the principal priority will be to preserve this species of monkey.

Magnanini, who at one point in his career was ready to pronounce the mico extinct (until his partner, Coimbra Filho, happily discovered one), says the mico had the misfortune to be best adapted to Brazil's humid Atlantic coastal region—where development of the country has been most intense.

● Brazil's Health Ministry has released partial results of its most intensive

survey to date on the incidence of Chagas Disease, and the picture is not very bright. Researchers found the ailment prevalent in 1,238 of 1,750 counties that were surveyed (Brazil has nearly 4,000 counties). These results led to estimates that 8 million people in this country may have the disease, either in its latent or acute form.

Chagas Disease is a Latin American malady for which there is no known cure. It is caused by a protozoan parasite known as *Trypanosoma cruzi* and is transmitted by an insect known colloquially as the "barbeiro" (Portuguese for "barber bug") or "chupao" ("sucker"). Chagas Disease, which attacks the heart, causes long term debility, often resulting in death. Most fatalities are among people 25-50 years old who come from rural areas.

The disease is transmitted in a bizarre way: "barber bugs" living in the thatched roofs or mud-brick walls of peasant farmers' shacks come out at night and bite people, usually near their eyes. Then the insect defecates into the bite, sending the dangerous parasite directly into the person's bloodstream. The Health Ministry survey showed that Chagas Disease is further spread through unsupervised blood transfusions—even in seemingly modern, advanced cities such as Belo Horizonte and Sao Paulo, where there are large colonies of farm migrants.

"In Brazil, most cases of Chagas Disease go unrecognised, because of a lack of good medical care in rural areas," declared Dr J. Romeu Cancado, head of the medical clinic at the Federal University of Minas Gerais Medical School. He added that the problem of Chagas Disease has been further complicated by failure to include a detailed study of the disease in leading Brazilian medical schools. Most medical researchers at present doing work on Chagas Disease are in fact non-Brazilians.

Asked about prospects for finding a cure for Chagas Disease, Dr Cancado said that several drugs had been pre-



sented recently as cures for Chagas Disease, but most had turned out to be too toxic or ineffective in humans.

● Brazil is seriously determined to cut its petroleum consumption (it had to import four-fifths of its crude oil in 1975, at a cost of around US \$3,800 million), and President Ernesto Geisel has asked petroleum researchers to develop an efficient Brazilian product that would contain 20% alcohol.

Brazil has great potential for producing alcohol. It is the world's biggest cane-sugar grower, and it has vast plantations of cassava root and sweet potatoes, which are thought to be alternative sources of extractable alcohol. The nation's present rate of alcohol production is 270 million litres a year. Based on figures for petrol consumption, Brazil would have to boost alcohol production to 3,000 million litres annually to meet the 20% goal.

Brazilian gasoline now contains about 5% alcohol. Some say that the alcohol mixture can be increased to 20% without lowering the efficiency of automobile engines, but Mario Gellini, an engineer with the Brazilian subsidiary of the Ford Motor Company, says that 15% is the maximum amount of alcohol that can be tolerated if cars are not to run badly.

Bruce Handler
Rio de Janeiro



Competition 5

As correspondents continue to point out, *Nessiteras rhombopteryx* is an anagram of "Monster hoax by Sir Peter S." or "Yes both pix are monsters, R." (for Rines). A prize of £10 is offered for the best anagram of any scientific concept. Closing date for entries is March 10.

FOR Competition 4 readers were invited to submit about 100 words culled from the pages of *Nature* 100 years hence. Entries from F. P. Hughes and S. Gilbert deserve special mention, but the winning entry came from R. Ternbach, of Cambridge, Massachusetts, who receives £10 for this submission:

The Lunar Observatory for Orthomolecular Nebulosity has surveyed one of the twelve equally spaced peculiarities recently shown to surround the galaxy at cosmological distances. Optical plates exposed through the moonstation telescope show a ring (head) with an arc (horns) above.

Gas clouds are ejected from twin singularities (nostrils) in the head at a rate of 10 min^{-1} (breathing rate). Glitches in the ejection rate (snort bursts) have a mean inter-snort interval of 1 sec. Absorption spectra of snort gases suggest a molecular composition strikingly similar to bovine breath.



Fig. 1 Appearance of the peculiarity on optical plate made at the Lunar Observatory for Orthomolecular Nebulosity.

SWEDEN

Making sense of senselessness

As progress falters in the drawn-out second round of the Strategic Arms Limitation Talks (SALT), concern is also growing about the implications of large-scale environmental warfare. But popular attention is often diverted away from both the true state of present technological developments and the strategically valueless damage which the military use of existing techniques has already done. From Stockholm, Wendy Barnaby reports.

WHEN two such prestigious organisations as the Royal Swedish Academy of Science and the Stockholm International Peace Research Institute (SIPRI) collaborate to produce a public statement on a topical issue, it is not surprising that it should attract a lot of publicity. But press reports of their predictions about war and the environment, which are contained in the latest edition of the Academy's journal *Ambio*, have generally been both inaccurate and misleading.

The journal describes the damage done to the biosphere by war, and the ways in which natural environment phenomena could be manipulated for belligerent purposes. It also discusses some of the international negotiations to draw up treaties prohibiting modification of ecological systems for hostile ends. Most of the publicity surrounding the issue has concentrated on articles which document the worldwide spread of all types of sophisticated weapons, describe the likely results of a nuclear war in Europe, and catalogue the possibilities of weather manipulation.

Press accounts of the prospects for environmental warfare have dwelt on seeding clouds, modifying hurricanes, oceans, earthquakes and climates, and tampering with the ozone layer, without mentioning what *Ambio* stresses: that the techniques for doing these things involve many practical difficulties and are at the moment "theoretical and speculative" only. Geophysical modification as a weapon system is certainly not just around the corner, but the presumption is that the public has the right to know which stage in the sequence has been reached: a *fait accompli* is harder to oppose than something not yet completed.

The global proliferation of modern conventional weapons suggests that more attention might be paid to the damage they can cause. Dr Malvern Lumsden, a Research Fellow at SIPRI, describes the impact of strategic bombing in the Second World War, for example of Berlin, and of the bombing

of South Vietnam by the United States. He concludes that the level of social organisation (which is different from the level of "development") of the bombed population is the prime determinant of its ability to withstand environmental stress. This conclusion is interesting in the context of the environmental stress that urban guerillas seek to inflict through the use of terror—a topic on which the journal might have shed some light by considering which factors most often influence the outcomes of sustained campaigns of kidnapping, hijacking and attacks against embassies.

Apart from anticipating future weapon systems, the press could also publicise the extent of the damage caused by those presently deployed. In another article, a former US marine officer, Professor Arthur Westing, describes the three prongs of American tactics in South Vietnam between 1965 and 1973. Professor Westing, presently a botanist who visited SIPRI as a researcher in 1975, details the effects on the country's ecology of high explosive munitions, herbicides and land-clearing tractors ("Rome ploughs"): the instruments used by the USA to make the land inhospitable to enemy guerillas. One quarter of the land area of the entire country was covered by B-52 crater fields alone. One tenth of the land area—about 1.7 million hectares—was sprayed once or more with herbicides. And about 2% of the land area was cleared by Rome ploughs, 33,000-kg armoured tractors whose blades can split and topple trees of almost any size. These destructive techniques proved to be of only doubtful military value, but Professor Westing forecasts that future combatants will intensify their use in order to raise their military pay-off. The result will be even more desolation than was caused in South Vietnam.

Destruction on the scale implied by present levels of armaments can, of course, only be supported by enormous resources. Another SIPRI Research Fellow, Ronald H. Huiskens, calculates that world military expenditure totalled \$210.300 million in 1974, and that the raw materials used for global military purposes are at present worth about \$250.000 million—the equivalent of the combined gross national products of the 65 countries of Latin America and Africa. That natural resources are used to desecrate the environment in which they were formed represents just one of the many ironies characterising the present ridiculous levels of militarisation. □

CERN

II on target

The Geneva-based European Organisation for Nuclear Research (CERN), in which 12 European governments participate in a collaborative programme of subnuclear physics research, largely through teams of visiting scientists, has two particle accelerators that have been operational for many years. Peter Collins reports on the super proton synchrotron (SPS) now under construction

THE meeting of the CERN Council just before Christmas marked the end of an era with the retirement of Professor W. Jentschke following his five-year term as Director General of CERN I, the original CERN laboratory. Since January 1 of this year the two CERN laboratories—CERN I and the one to house the SPS, CERN II—have been combined. Instead of two separate Directors General, the whole complex has two men working side by side: Dr J. B. Adams, as Executive Director General, is responsible for everything concerned with management, while Professor L. van Hove of Belgium fills the new appointment of Director General responsible for research.

Interest at CERN inevitably centres, however, on the 400-GeV SPS. Unlike many (and perhaps most) such building projects, its construction is moving ahead on schedule and within its estimated budget. The last of 744 bending magnets in the 6.9-km ring was installed a month or so ago to coincide with the meeting of the CERN Council. According to Dr Adams, who was the Director General of CERN II, there have been few serious problems during construction of the new machine.

Apart from the apparently inevitable delays arising from late delivery of components, only one major difficulty seems to have arisen. This was the discovery, early in 1975, of serious deterioration in the insulation of a number of the magnets that had already been installed. Urgent and intensive detective work showed that the failure was always in the same area of the coils: it was found to be caused by the use, on the ends of cables, of a cleaning fluid that contained a high proportion of phosphoric acid. Where this had not been completely cleaned off, the acid attacked the resin used for insulation, and had the effect of continuing its "curing". The result was that the resin hardened, shrank, and eventually cracked, allowing the acid to get through into the glass cloth used for the inner insulation, and thence to the

THE annual review of the Christian names of the "top children" whose birth are announced in the London *Times* appeared as usual in the New Year. For the boys, James, Thomas and Nicholas headed the list, with, for girls, Sarah, Emma and Alexandra in the corresponding positions. This year the list has stimulated an interesting correspondence on the sociological problems relating to the choice of what are perhaps more accurately described by the American term "given" names. No *Times* reader apparently calls his child Tracy or Charlene, though there is a growing tendency to saddle girls with classical names like Corinna, Flavia, Gratia and Xanthe. One writer, from the Savage Club, calling the list "astonishingly class distinctive", recommended the introduction of a Christian Name Discrimination Act, banning socially-divisive names and having, like the French, an approved list from which names must be drawn. This last course has given difficulties to Breton nationalists who wish to use names of folk heroes who were not recognised by Napoleon.

Names may be as interesting to the parasitologist as to the sociologist. In 1939 I became involved in the problem of lousiness in English children. Before the war official statistics suggested that the head louse was almost extinct, for only one or two per cent of children were detected with the parasites by the officials of the School Medical Service in their routine school inspections. However, when town children were evacuated to the country to escape the risk of bombing in September 1939, far more than had been expected were found to be verminous. The Board of Education, as it then was, defended its statistics, and suggested that the children had become infested during the long summer holiday. Political arguments arose. Socialist education committees in towns accused Tory reception areas of exaggeration. No one seemed able to produce the facts

which had led to what was rapidly becoming an ugly situation, particularly as there was as yet no enemy bombing to distract people's attention.

As no one else seemed to be trying to find out the facts, I approached

Names to remember



KENNETH MELLANBY

the Board of Education with a plan. To the Board's credit, I was given every encouragement and £200 to investigate. I found that, in our cities, over half the girls between 5 and 12 years old were lousy, and that a third of the boys were similarly affected. There was no evidence that the rate of infestation rose during the holidays. It was clear that most infestations were not being detected at routine inspections.

These results were all published with the approval of the Board of Education and the Ministry of Health, though they were clearly critical of work of those government bodies. My paper was censored only

in very minor ways. I was asked to omit a section where I identified the incidence of infection in children who were registered as belonging to different religions and different Christian denominations. I agreed that this information might exacerbate inter-sectarian quarrels. But I was also asked to cut out a section on Christian names, which was thought to be too frivolous for wartime publication, and I think that the information may now be revealed.

In one city which had a particularly high rate of lousiness (70 per cent of all ten year old girls) I noticed that children named after film stars, children with saints' names and children with the names of the Royal Family were the most likely to be lousy. Marlene (often spelt Marleen), Shirley, Bernadette, Margaret, Rose, Marina—they always warned me in advance what to expect.

This, of course, brings us back to the sociological field. Lousiness tended to be highest in large families, where reinfestation from siblings even after successful treatment was common, and where individual attention by the mother was less effective than when she had fewer children to care for. It has been suggested that children named after film stars or royalty come from families with less of a tradition than those where the same few names are used generation after generation, and where parental care may be greater. This may be the explanation. But I suggest that anyone studying sociological problems relating to names bears these observations in mind. During the war I found them useful. Although the results had to be confirmed by individual examinations, I could make a good estimate of the lousiness of a class of school children simply by reading through the register. As the head louse is now staging a comeback in some British towns, it will be interesting to see whether it still prefers to infest the holders of any particular names.

coils themselves. The insulation system itself then became a conductor.

Every one of the 300 or so magnets already assembled was examined, and dismantled, repaired or rebuilt where necessary. As an extra precaution, further insulation in the form of two layers of kapton foil was wrapped round each coil, and it was found possible to do this with those magnets which had not deteriorated, but had already been installed. Every one of the 744 magnets in the ring has been treated in this fashion.

The fact that nearly 300 of these big

magnets were rebuilt within the SPS Assembly Halls, without delaying the very strict construction schedule and using CERN's own staff and facilities, is an indication of the efficiency and skill with which the whole project is being handled. Tested under conditions with peak fields equivalent to the 400-GeV energy level for which they are intended, the magnets have shown no further failing, nor do there seem to be any serious problems with any of the other components.

Apart from some delays in delivery of magnets for the beam transfer lines

to the experimental areas, the rest of the construction is well on target, and the accelerator is expected to be operational in the latter part of 1976. This is perhaps no more than was to be expected from an establishment that runs as sweetly as CERN. To many it is an object lesson not only in international scientific and technical cooperation but in the noticeably pleasant atmosphere and evidently high staff morale as well. It could well be studied by certain international agencies of the UN family a few miles away in Geneva itself. □

correspondence

Proceedings against professor

SIR,—In *Nature* recently under the heading 'Anti-nuclear critic faces dismissal' (October 23, 1975, page 636) it was maintained that Professor Jens Scheer is "head of a university department" and is threatened with dismissal apparently because he criticises the construction of nuclear power stations. Both statements are incorrect.

(1) The "head" of our department is elected democratically each year by the professors, students and technical personnel. Professor Scheer has never held this office, which is at present occupied by Horst Diehl.

(2) It is true that various disciplinary proceedings have been initiated against Professor Scheer but the reason for these measures is neither his criticism of the West German nuclear programme, which is shared by a number of his colleagues against none of whom have any such proceedings been taken, nor simply his membership of what we consider a rather insignificant Maoist group.

Your readers might infer from the article that the West German authorities disregard individual rights and use improper methods in order to silence their critics and that the true reasons for the initiation of disciplinary proceedings against Professor Scheer are not those which had been stated officially. Such an inference would be wholly unfounded; readers should be aware of the following facts.

On two separate occasions, Professor Scheer was involved, with others, in violent activities at the University of Bremen: in October 1973 a meeting of Christian Democrat students was broken up by force, and in September 1975 a press conference of the Students Committee was pelted with eggs and tomatoes. In both cases the university took out disciplinary proceedings against Professor Scheer. The earlier case, in which the Rector imposed a fine on Professor Scheer, is under appeal; the latter is still under consideration. Professor Scheer's action in 1973 also led to the District Court of Bremen sentencing him to three months imprisonment (suspended) and a fine of DM3,000. This too is under appeal.

Action has also been taken against Professor Scheer by the Senate of the Free Hanse Town of Bremen under the provisions of the Bremen Civil Servants Law. Since December 9, 1975, he has been under suspension on half

pay as a result of his alleged anti-constitutional activities.

Yours faithfully,

STEFAN VON AUFSCHNAITER
(Conrektor of the University)
SIEGFRIED BOSECK
HORST DIEHL
WOLFGANG DREYBRODT
DIETER VON EHRENSTEIN
KLAUS HAEFNER
PETER RYDER
HELMUT SCHWEGLER
WOLF SIEGERT
(Professors of Physics)

University of Bremen,
Bremen, West Germany

Naming the blue-greens

SIR,—Recently, in several publications, organisms hitherto known as blue-green algae have been called "blue-green bacteria". It is appropriate to consider the popular name of the Cyanophyceae in a scientific journal since words, especially those used in science, should have clearly defined meanings and should not be subject to passing whims or personal preferences.

Cohn, more than a century ago, associated the apparently non-nucleate blue-green algae with the bacteria in the division Schizophyta (*Beitr. Biol. Pflanz*, 1, 141–207; 1875). Chatton subsequently distinguished the characteristic cellular organisation of these types of organisms as "procaryotic", (*Titres et travaux Scientifiques*, Sète, Sottano, 1937), and on this basis Stanier and van Niel proposed a major taxonomic dichotomy among living organisms, establishing the Prokaryota and Eukaryota (*J. Bact.*, 42, 437–466; 1941). Although the Prokaryota are essentially the same as the Schizophyta, rules of priority have evidently been waived and this distinction has become generally accepted. The eighth edition of *Bergey's Manual of Determinative Bacteriology* (Williams and Wilkins, 1974) refers to "the blue-green algae" as "allied to bacteria and at a level of equal importance . . . to the remainder of the Kingdom" (that is the Prokaryotae). But this is not to say that they are to be considered as bacteria. If we were now to start referring to cyanophytes as 'blue-green bacteria', we would implicitly synonymise the words 'prokaryota' and 'bacteria'. To what useful end?

It is generally accepted that living organisms should be named according

to one of three texts: the International Rules of Botanical, Zoological and Microbial Nomenclature. If a creature is to be 'moved' from one kingdom to another, its whole taxonomic status has to be reviewed and may have to be revised. If those of a whole class were to be involved, this would entail a lot of extra, and in my opinion, unnecessary work and headaches.

The word 'algae' has no clearly defined taxonomic meaning. It is a useful and convenient term for most non-vascular aquatic plants capable of photosynthesis with O₂ evolution. If the Cyanophyceae were to be shifted, lexicographically, from the algae to the 'bacteria', it would be necessary to change many other generalisations to conform with this transfer. For example, the primary producers which evolve O₂ would include 'bacteria' as well as green plants. The photosynthetic 'bacteria' would include aerobes as well as anaerobes, would contain either chlorophyll *a* or bacteriochlorophylls in thylakoids or associated with other subcellular elements, and would fix CO₂ either with or without O₂ evolution. Many of the seaweeds which often dominate tropical shores would have to be regarded as 'bacterial' growths. What would we gain by such verbal changes?

Unlike non-photosynthetic prokaryotes, blue-green algae rarely cause disease or decay, and thus relatively little money is spent on them. (Anyone who questions this should compare the budget of a bacteriology department with that devoted to the study of blue-green algae.) But although cyanophycologists are in a less fortunate position than bacteriologists when it comes to funding for research, personal remuneration, or public support, they should not seek to better their lot by calling themselves blue-green bacteriologists. Scientists should not use words misleadingly for base ends. In spite of the fairly evident and generally accepted phylogenetic homogeneity of the Prokaryota, the distinction between the classes are worth retaining. Although we may now handle them by similar technologic, yeasts are fundamentally fungi, cells from tissue cultures remain basically animal or plant cells, and blue-green prokaryotes are, by accepted standards, algae.

Yours faithfully,

RALPH A. LEWIN
*Scripps Institution of Oceanography,
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news and views

Spacing out the histone genes

from Benjamin Lewin

THE organisation of clusters containing many identical copies of a gene(s) is among the more intriguing features of eukaryotic DNA. The first such cluster to be identified was that of the genes coding for ribosomal RNAs. Assisted by the ready isolation of ribosomal genes in the form of the amplified nucleolar DNA of *Xenopus laevis*, analysis of the rDNA has shown that the pattern of organisation takes the form of a repeating series of transcription units, each representing a precursor molecule containing the sequences of both major rRNAs, adjacent units being separated by a region of DNA that is not transcribed. The transcriptionally inactive DNA separating the sequences coding for the precursor RNA is often termed the non-transcribed spacer. Because the precursor is itself somewhat larger than the total length of both mature RNAs (the length of the excess varies with the organism), the part of the precursor that is discarded is sometimes known as the transcribed spacer; its sequence is invariant and it most probably functions at some step after transcription. One of the most striking features of the rRNA gene cluster is that in spite of mutational pressure the copies of each gene seem to be identical. This implies that some mechanism must exist for suppressing variation among the gene copies and this may be the role of the non-transcribed spacer.

The histone genes comprise another system of clustered repetitive genes. The histone genes of the sea urchin are all repeated several hundred-fold in the genome, and similar repetition is also found in other organisms to a varying degree. Because their base composition differs from that of the bulk of the genome, the histone genes can be isolated as a satellite fraction of distinct buoyant density. This in itself suggests that they are clustered into one or a small number of groups. Two types of arrangement might be possible—separate clusters each representing many copies of one of the five histone genes or a single type of cluster containing all five genes. Two recent series of experiments have demon-

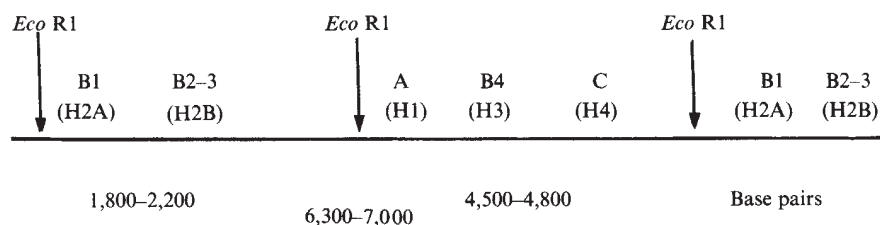
strated the existence of a single type of repeating unit and also have implied that it may possess a spacer region(s) longer than the total coding length of the five genes.

These experiments were made possible by the fact that the restriction nucleases *Hin* III and *Eco* R1 make a limited number of cuts in what can be inferred to be a simple repeating unit. Because such a large amount of histone DNA is present in the sea urchin genome, its cleavage products can be identified directly without the need for extensive purification of the starting material. Kedes *et al.* (*Cell*, **6**, 359–369; 1975) showed that when the DNA of *Strongylocentrotus purpuratus* is treated with the enzyme *Hin* III, a single major fraction is present upon centrifugation through a sucrose gradient; this represents histone DNA and sediments at 17S. Treatment with *Eco* R1 nuclease generates two major fractions, sedimenting at 14S and 11S. Starting with partially purified histone DNA (5–30 fold enriched), Weinberg *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 4815–4819; 1975) characterised the restriction products upon acrylamide gels. *Hin* III generates a single fragment with a mobility suggesting a length of about 6,300 base pairs; *Eco* R1 produces two fragments, with mobilities corresponding to 1,800 and 4,500 base pairs. Only these fragments hybridise with histone mRNA; their gel mobilities are consistent with the sucrose sedimentation rates. The model immediately suggested by these results is that histone DNA consists of a single type of repeating unit, which happens to have a single site recognised and cleaved by *Hin* III and to have two sites that are subject to attack by *Eco* R1 nuclease.

By mixing *S. purpuratus* DNA cleaved by *Eco* R1 with the *Eco* R1-treated DNA of plasmid pSC101, Kedes *et al.* (*Nature*, **255**, 533–537; 1975) isolated hybrid plasmids carrying random parts of the sea urchin genome. Hybrid plasmids carrying histone genes were identified (after cloning in *E. coli*) by hybridisation with labelled histone mRNA. Two plasmids carrying histone

genes were characterised: pSp2 includes a DNA fragment estimated at 4,400 base pairs by gel electrophoresis and pSp17 carried a fragment of 1,700 base pairs. When Kedes *et al.* (*Cell*, *op. cit.*; 1975) investigated the nature of these histone gene sequences, they found by electron microscopy of heteroduplexes that pSp2 and pSp17 are homologous only along the 8,700 base pair length of the pSC101 sequence itself. The contours of the single strand loops give more accurate assessments of the lengths of the eukaryotic fragments, 4,800 base pairs in pSp2 and 2,200 base pairs in pSp17. Hybridisation experiments confirmed that the pSp2 plasmid carries the sequence of the 14S fragment cut from histone DNA by *Eco* R1 whereas the pSp17 plasmid represents the 11S sequence. The lack of homology between the 4,800 and 2,200 base pair sequences supports the idea that the repeating unit of histone DNA has been cut into two parts.

Which histone genes are represented on these two parts of the repeating unit? Histone mRNA can be isolated from the polysomes of developing sea urchin embryos as a 9S fraction and the mRNA of *S. purpuratus* has been separated into several classes on acrylamide gels. Two of these classes, A and C, have been shown to code for histones H1 and H4 respectively (Levy *et al.*, *Cell*, **4**, 239–248; 1975). The classes B1, B2, B3 and B4 together code for H2A, H2B and H3; the individual classes have not been equated with particular histones, but comparison with the template activities of the corresponding classes in another sea urchin, *Lytechinus pictus*, suggests tentatively that B1 may code for H2A, B2–3 for H2B, and B4 for H3. When these messenger fractions were hybridised to the hybrid plasmids Kedes *et al.* found that A, C and B4 annealed exclusively with pSp2 whereas B1 and B2–3 annealed only with pSp17. Using histone mRNA fractions separated in the same way, Weinberg *et al.* obtained precisely the same results with the *Eco* R1 histone DNA fragments separated on acrylamide gels. This suggests a map for the histone genes:



Two features are implicit in this map; it represents a regular repeating structure containing all five histone genes; and it must have appreciable spacer regions since the total length needed to code for all five histones is only 2,100 base pairs. This organisation obviously raises the question of whether the histone genes are transcribed as a single unit, presumably cleaved later into the individual messengers that represent the only form in which template activity for histones has been identified. Histones H2A, H2B, H3, H4 are found in equimolar amounts in chromatin, which makes their coordinate gene expression an attractive model, but H1 is found in smaller amounts. No precursor to histone mRNA has been identified, but it is interesting that such a model would imply that at least some of the spacer is transcribed. What is known about the sequences of the histone messengers and genes suggests that there cannot be much variation in the copies of each gene; probably they are all identical. This is subject to the caveat that although H2A, H2B, H3 and H4 all display unique amino acid sequences, some variation is found in H1, which may therefore be represented by more than one type of gene; how the H1 genes might be arranged and controlled within the repeating cluster is a matter

of some interest. In contrast to the apparent homogeneity in the copies of each histone gene in a given sea urchin species, Grunstein *et al.* (*Cold Spring Harbor Symp. quant. Biol.*, **38**, 717-724; 1973) noted that there is only limited homogeneity between the corresponding histone genes in different sea urchin species (in spite of the common amino acid sequences of the corresponding proteins); this emphasises the evolutionary stress that must be placed on conservation of sequence within a species. What is responsible for the maintenance of identity among the gene copies? It is tempting to speculate that some of the regions which do not code for histones might represent non-transcribed spacer, in which case a common type of structure might be proposed for both the rRNA and histone gene clusters, with a non-transcribed spacer perhaps implicated in helping to suppress variation. Of course, it is also possible that the unidentified regions of the histone gene cluster may code for other proteins or represent transcribed spacer. Important future experiments therefore will be to define completely the location of each histone gene in the repeating unit and to investigate the sequence organisation of the remaining sequences, and to determine whether they represent transcribed or non-transcribed regions.

The scene for the recent reports was set a few years ago by De Maeyer and his colleagues who boldly applied RNA, extracted from either mouse or monkey cells which had been induced to synthesise interferon, directly to heterologous cells and showed that the recipient cells were able to make murine or simian interferons, respectively (De Maeyer-Guignard *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1203-1207; 1972). The amount of interferon made was quite small (not more than 30 units) perhaps due to the probable inefficiency of exogenous mRNA when applied directly to cells.

The obvious extension of this work has now been reported by De Maeyer and his coworkers (Thang *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3975-3977; 1975) who have translated RNA extracted from induced mouse cells in a wheat germ cell-free system to yield an antiviral product with the properties of mouse interferon. About 700 units per μ g added RNA were made. Similar results have also been described for RNA extracted from human cells in cell-free extracts from mouse ascites cells and rabbit reticulocytes (Pestka *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **72**, 3898-3901; 1975; Reynolds *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4881-4885; 1975).

In view of these findings it is naturally to be expected that if such RNA preparations were to be injected into frog oocytes then translation of the exogenous mRNA would occur. That this is indeed the case has been demonstrated by Reynolds *et al.*, (*op. cit.*) who have achieved interferon titres of 100,000 units in homogenised oocytes after a 24 h incubation. Moreover, when 3 H-leucine was included in the incubation and the product partially purified, using either immunoprecipitation with anti-interferon antibody or by affinity chromatography on a concanavalin A-agarose column, then subsequent electrophoretic analysis on polyacrylamide gels yielded a distinct peak of radioactivity with mobility corresponding to a molecular weight of 25,000 (this is about the expected value for human interferon). When RNA from cells not induced to make interferon was injected into oocytes very much less radioactivity was found in this peak. These results are striking when one considers that the injected RNA was the total mRNA fraction (containing poly(A)) from the induced cells and that the messenger for interferon could only comprise a very small proportion of this.

It seems likely that interferon is the first biologically active eukaryotic protein to be synthesised in a cell-free system dependent on added mRNA. But where does this get us? First it

Interferon translated

from David Metz

INTERFERON is the antiviral protein synthesised by animal cells following infection with viruses. Since it is a cellular protein one would naturally suppose there to exist a corresponding mRNA species which could be translated in a cell-free protein synthesising system to yield, one would hope, the biologically active antiviral molecule. Three recent papers confirm this expectation.

The demonstration of the translation *in vitro* of interferon mRNA is made possible by the remarkably high biological activity of interferon. Although it has not yet been purified to unequivocal homogeneity, it is clear that the specific activity of interferon

must be at least 10^9 units of biological activity per mg protein. Thus the synthesis of exceedingly small amounts, by weight, of the active macromolecule may still be detectable by bioassay in which the reduction of virus yield is determined. A second convenient property of interferon is that of species specificity. Thus, for example, mouse interferon is active on mouse cells and not on human cells. Consequently if mRNA for human interferon is translated in an *in vitro* system prepared from mouse cells, one is able to verify that the antiviral activity synthesised is coded by the exogenous human mRNA and not by any endogenous murine messenger.

should enable the synthesis of interferon and its regulation to be studied in the intact cell since the mRNA as well as its product may be quantitated. Furthermore, *in vitro* synthesis of interferon should permit one to approach the question of the role of carbohydrate substitutions in interferon activity. Interferon has long been known to be a glycoprotein but whether the glycosylation is essential for activity remains unclear (if it is, then it must be occurring in the *in vitro* systems.)

Second, the apparent efficiency of the oocyte in translating interferon mRNA may assist in the determination of the primary structure of the protein, especially if the messenger can be at all purified from the other cellular mRNAs. The acquisition of sufficient pure interferon for amino acid sequencing by conventional techniques would be a formidable undertaking (though it is being attempted; Anfinsen *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3139-3142; 1974) since the very high specific activity implies the need for

very great volumes of comparatively low titre starting material. The ability of the oocyte to synthesise interferon highly labelled in various amino acids may well expedite the task of determining the primary structure. If this were known then chemical synthesis of the molecule could be attempted. If this in turn was achieved then it might, because of the very high specific activity, prove to be a more economic way of preparing the quantities of interferon required for clinical trials than the cell cultures now used. □

The A, B, C viruses

from Arie J. Zuckerman

VIRAL hepatitis now constitutes the main hazard of transfusion of blood and certain blood products. It was estimated in 1972, for example, that transfusion-associated hepatitis caused more than 30,000 cases of overt hepatitis and 1,500 to 3,000 deaths each year in the United States (*US Department of Health, Education and Welfare*, 1972). Since there are many sub-clinical cases of viral hepatitis the actual incidence in the USA has been estimated as high as 150,000 cases annually.

The existence of two types of viral hepatitis in man, infectious or epidemic hepatitis (hepatitis A) and serum hepatitis or homologous serum jaundice (hepatitis B) has been recognised for many years, and there is substantial evidence that these two types of infection are caused by antigenically distinct viruses. But it was difficult to distinguish clearly between these forms of hepatitis in the absence of specific laboratory tests. Differences were based principally on epidemiological observations and transmission experiments to human volunteers, faecal-oral transmission and an incubation period of about one month in the case of hepatitis A, and essentially tissue penetration and a long incubation period of up to six months in the case of hepatitis B.

The discovery of hepatitis B surface antigen (Fig. 1) and its association with infection has led to the development of a bewildering array of tests for screening blood donors and blood products. It was soon firmly established that blood containing this antigen was associated with a high risk of hepatitis and that such blood should not be used for transfusion. It rapidly became apparent, however, that the elimination of antigen-positive blood reduced post-transfusion hepatitis type B by about only 30% in some areas (*WHO Techn. Rep. Series*, No. 512, 1973). Although

the more recent introduction of sensitive methods of detection such as radioimmunoassay reduced further the incidence of post-transfusion hepatitis, cases of hepatitis B and non-B infections continue to occur (Koretz *et al.*, *Lancet*, **2**, 694; 1973). These remaining infections were generally ascribed to still insufficiently sensitive assay techniques for hepatitis B (Hollinger *et al.*, *New Engl. J. Med.*, **289**, 385; 1973 and *ibid*, **290**, 1104; 1974) and to hepatitis A, which can also be transmitted by blood.

The identification by immune elec-

tron microscopy of virus-like particles (Fig. 2) in faecal extracts of patients with hepatitis A (reviewed in *Nature*, **252**, 193; 1974) and in experimentally infected marmosets and chimpanzees, provided an antigen which could be used for serological tests for hepatitis A antibodies and thus for evidence of infection. Immune electron microscopy has been used widely but immune adherence haemagglutination, complement fixation and radioimmunoassay techniques have been developed and are being applied on a limited scale. Sera from patients with hepatitis B-negative post-transfusion hepatitis in the United States were recently examined for evidence of infection with hepatitis A virus, and although relatively few patients have been tested so far sero-

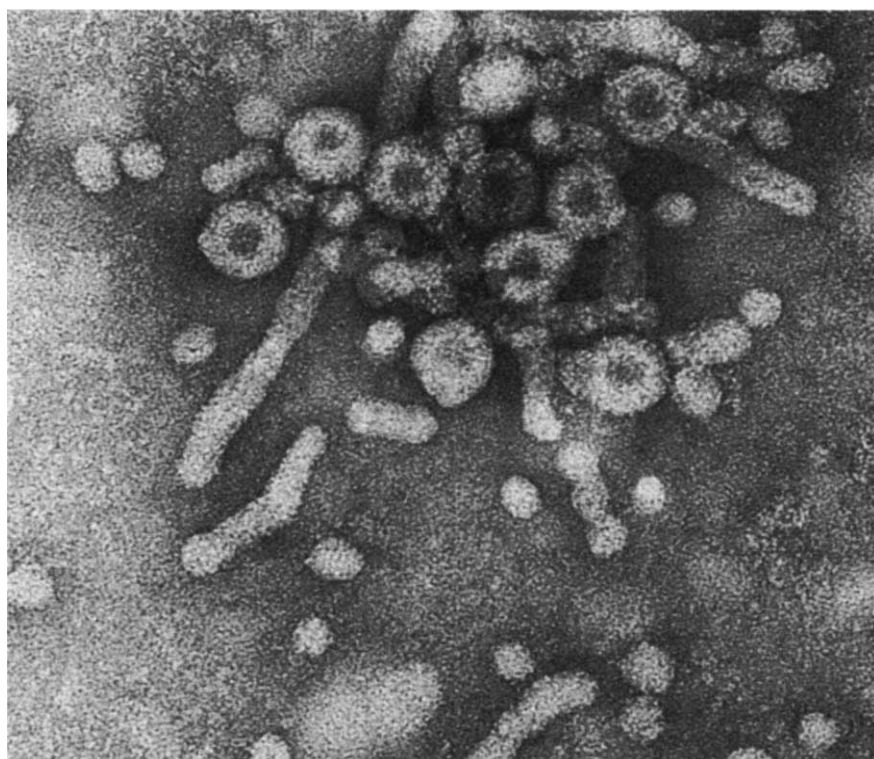


Fig. 1 The morphology of hepatitis B antigens showing three distinct entities, small 22 nm spherical particles, tubular forms of varying length and a large 42 nm double-shelled spherical particle. Original magnification $\times 252,000$. (Reproduced with permission from *Human Viral Hepatitis*, North Holland and American Elsevier, 1975.)

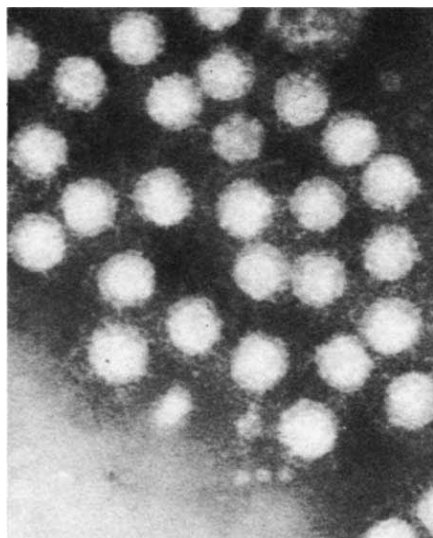


Fig. 2 Virus-like particles measuring 27 nm in diameter found in faecal extract from a chimpanzee infected with human hepatitis A. Original magnification $\times 275,000$. (Electron micrograph from a series by A. Thornton, A. J. Zuckerman and J. D. Almeida.)

conversion to hepatitis A was not found in any of these patients (Feinstone *et al.*, *New Engl. J. Med.*, **292**, 767; 1975). A new term was coined; non-A : non-B hepatitis.

Feinstone and his colleagues were unable to implicate infection with cytomegalovirus or Epstein-Barr virus, which are known to induce liver damage as part of the generalised infection caused by these herpes viruses. Recently, Alter and his associates (*Am. J. med. Sci.*, **270**, 329; 1975) pointed out that in the United States when blood obtained from volunteer donors is pretested by radioimmunoassay for hepatitis B surface antigen approximately 90% of the remaining cases of post-transfusion hepatitis are serologically unrelated either to hepatitis A or hepatitis B viruses. It was considered, therefore, that a previously unrecognised human hepatitis virus may exist. This agent may be hepatitis virus C, a term introduced by Prince and his colleagues (*Lancet*, **2**, 241; 1974). They noted that an agent other than hepatitis B was the cause of 71% of 51 cases of post-transfusion hepatitis identified during a prospective serological study of 204 patients in New York. The incubation period was relatively long and the clinical and epidemiological features of the infection were not consistent with hepatitis A.

It is thus evident from these studies that a hepatitis virus C may indeed exist, although the precise criteria which would be virologically acceptable for a new infective agent have not yet been satisfied, despite the application of a battery of modern virological techniques. □

Wigner cusps in nuclear reactions

from P. E. Hodgson

WHEN a proton is incident on a nucleus, many different reactions can take place. It may be elastically or inelastically scattered, it may be captured to form a compound nucleus which subsequently emits neutrons, protons or other particles, it may undergo (p,n) charge exchange, it may pick up a neutron to form a deuteron, it may knock out an alpha particle and so on. The relative probabilities of these processes depend on how easily the particles can get in and out of the nucleus (barrier penetration factors) and on the structure of the nucleus. The penetration factors depend on energy, and as the energy of the incident particle increases more and more reactions become possible.

At low energies the compound nucleus processes dominate, and then the cross section for all the non-elastic reactions depends on the penetration factor for the incoming particle, and this increases smoothly with energy.

As this energy increases this cross section has to be shared out among more and more of the reaction processes. The situation is rather like steadily increasing the flow of water into a closed tank, and at the same time making more and more holes of different sizes in the side of the tank. If we then concentrate our attention on the water coming out of one particular hole, we still see that at first it increases because of the increasing flow of liquid into the tank, and then decreases because more and more of the water is flowing out of all the other holes.

If we look very carefully, we may notice another effect: the flow of water out of our one hole falls slightly just after another hole has been made. This is because at any one time the total flow out must be the same as the total flow in, since the tank is closed and water is incompressible.

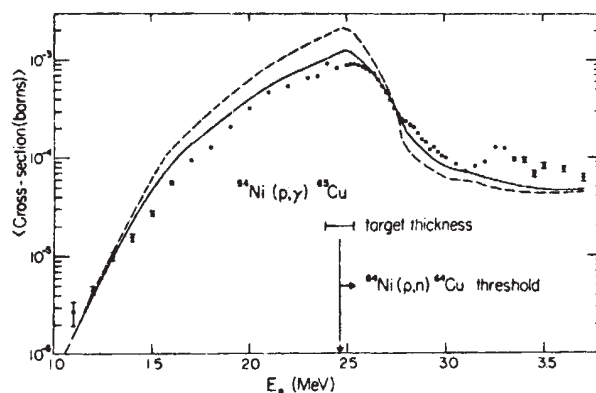
A very similar effect in nuclear reactions was predicted by Wigner in 1968 (*Phys. Rev.*, **73**, 1002). Each reaction process has a threshold energy: if the incident particle is below this energy the reaction cannot take place, but as soon as it is exceeded the cross section increases rapidly from zero. (This is the counterpart to punching another hole in the tank.) But since the total flux is constant, this must produce a small reduction in the cross section for all the other processes, and Wigner calculated the shape of the resulting cusps in the cross section as a function of incident energy.

It is interesting to try to observe these cusps as they would provide a delicate test of nuclear reaction theory but they are not easy to see. Usually there are so many hundreds or thousands of reactions taking place at the same time that the opening of another reaction channel, as it is called, does not make any perceptible difference.

The best conditions for observing Wigner cusps occur in the reactions of light nuclei, for in this case the level density is smaller and hence there are fewer channels. The effects of opening another channel are then more prominent, and indeed several cusps have been observed in light nucleus reactions, associated with the crossing of thresholds in neutron-induced reactions.

It is also interesting to look for Wigner cusps in proton reactions on heavier nuclei, and a remarkable example has recently been found in the $^{64}\text{Ni}(p,\gamma)^{65}\text{Cu}$ reaction by Mann and his colleagues at the California Institute of Technology (*Phys. Lett.*, **58B**, 420; 1975).

This reaction was chosen because even though it is on quite a heavy nucleus it provides especially favourable conditions for the observation of



Excitation function for the $^{64}\text{Ni}(p,\gamma)^{65}\text{Cu}$ reaction showing the Wigner cusps due to the crossing of the neutron threshold at 2.46 MeV. The curves show the results of Hauser-Feshbach statistical model calculations with (full) and without (dashed) the width fluctuation correction.

a Wigner cusp. These are that the new channel opens quickly, absorbing an appreciable fraction of the total flux, and that there are very few channels open already to share the resulting decrease in flux. These conditions are very well satisfied for the (p,γ) channel on ^{64}Ni at the opening of the neutron threshold. The lowest excited states of ^{64}Ni are at relatively high energies and this, together with the low energy of the neutron threshold (opening of the (p,n) channel), reduces the contribution of the inelastic scattering and compound elastic processes to the total reaction cross section. A possible competing reaction, alpha particle emission, is strongly inhibited by the Coulomb barrier, so that the total reaction cross section below the (p,n) threshold is mainly composed of the (p,γ) channels. Another favourable feature is the high density of low-lying states in ^{64}Cu , the final nucleus in the (p,n) reaction, which ensures that the total (p,n) cross section increases very rapidly, producing a strong depletion in the (p,γ) cross section.

The measurements of the (p,γ) cross section were made from 1.1 to 3.7 MeV in 100 keV steps, and the yields for the reaction to the first three excited states of ^{64}Cu were summed to give the results shown in the figure. It is notable that the cross section increases steadily from 1 to 2.5 MeV and then drops dramatically by a factor of 10 over the next 0.5 MeV as a result of the opening of the (p,n) thresholds. In this case the Wigner cusp is not a tiny perturbation but a major feature of the cross section. Many neutron thresholds are passed above 2.5 MeV, and these all contribute to the drop in the (p,γ) cross section.

A small resonance is visible in the (p,γ) cross section at about 3.27 MeV. This is attributed to the state in ^{64}Cu that is the analogue of the first excited state of ^{65}Ni .

At such low energies the reaction is dominated by the compound nucleus process; the incident proton is captured by the ^{64}Ni nucleus to form a compound nucleus ^{65}Cu and after a long time on the nuclear scale it emits one or more gamma rays until it returns to the ground state. The cross sections for such processes can be calculated by the Hauser-Feshbach statistical theory, using barrier penetration factors calculated from the appropriate optical model potentials. Since the number of open channels is small, this reaction is a severe test of the theory.

The results of Hauser-Feshbach calculations are shown by the dashed curve in the figure, and are in qualitative agreement with the measurements. If account is also taken of the correlations between the partial widths in the

entrance and exit channels by including the width fluctuation correction the improved results shown by the full line are obtained.

This comparison shows that the Hauser-Feshbach theory is well able to account for the Wigner cusps, and that it is important to include the width fluctuation correction. $\square$

Disappearing habitats

from Peter D. Moore

It is believed that the Romans were responsible for the construction of an extensive series of draining channels in the low-lying coastal area between Newport and Cardiff in South Wales, commonly known as the 'Monmouthshire levels'. The value of this land, mainly for pasture, is maintained by a careful control of the water table, which has long been effected by these drainage ditches, locally termed 'reens'. The reens have an additional value in that they represent a diverse series of habitats for aquatic plants and animals and serve as an extensive refuge for a large number of plant and animal species.

Recently this area has come under threat from a number of quarters and an ecological survey of the area and the various interrelationships between such physical factors as water table and plant and animal communities was urgently required. Just such a survey has been conducted recently by P. M. Wade of the University of Wales Institute for Science and Technology, Cardiff, and some of the conclusions were presented during a recent meeting of the British Ecological Society in Cardiff.

The floristic diversity of the reens has been confirmed by Wade's study, in which he recorded over 400 plant species associated with the waterways and their adjacent hedges and meadows. He feels, however, that recent land use changes in the area are combining to reduce this diversity.

The drainage channels are arranged in a rectangular grid system, where subsidiary reens coalesce into main reens which ultimately discharge their load into the Bristol Channel through simple, one-way sluice gates. These gates are not always perfectly efficient and there is an occasional movement of estuarine water back into the reens, which serves to diversify yet further the habitats within these channels. The fields which are bordered by the reens are drained by small furrows on their surface, from which run-off water reaches the reens.

Recent land-use changes have included the installation of sub-surface drainage pipes which have resulted in a more efficient water-ridding system and a consequential improvement of the meadows for pasture. This has meant that field size can be increased by reducing the number of reens, either by allowing them to silt up or by actively filling them. It has also allowed an increase in stock density to take advantage of the improved pasture yield.

Historically, the reens have served the subsidiary function of providing water for the resident cattle. This practice has also brought its problems, because the trampling of stock around the ditches has damaged banks and increased the rate of silting. An increased stock density, of course, makes this an even more serious problem. An answer has been found in the provision of water troughs situated in the centres of fields which attract animals away from the ditches.

At this stage a farmer is bound to ask the question whether he needs the reens any more. Their maintenance is expensive; at one time they were dug out by hand, but now excavation can be carried out mechanically. This, however, has involved the removal of most of the accompanying hedgerows which themselves provided food and shelter for wildlife. The growth of emergent aquatic plants can be controlled by spraying herbicides and this reduces the need for frequent ditch clearance, but spraying is itself an expensive process particularly because of the labour involved. So, since the reens are no longer useful as a water resource for livestock, it seems advantageous to lower the water levels within them or even to empty them completely. The use of mechanical pumps to replace the earlier crude sluice system has now made this possible.

Inevitably these changes have become more widespread until the traditional management of the reens has practically disappeared and subsequently the character and the biotic richness of the habitats which they represented is also vanishing. At this point one can predict that naturalists and wildlife conservationists will begin to muster and cry out for the retention of the old system in at least a portion of the area occupied by these low-flying levels. It is not difficult to summon up sympathy for such views, especially since they emanate from a part of the country where man's recent activities have reduced biological diversity to an extraordinarily depauperate level. The remaining fragments must assume a particularly high status in the eyes of those members of the local populace who appreciate such things. One cannot help reflecting, however, upon the

innate conservatism of mankind and one wonders whether the Romans were faced by a similarly militant Celtic environmental lobby when first they dug the ditches. □

The EISCAT project

from G. N. Taylor

THE recent announcement that the UK Science Research Council is joining EISCAT draws attention to some significant current developments in upper atmospheric research. EISCAT stands for the European Incoherent Scatter Organisation, an association of research organisations from six countries which has been set up to build and operate a large new radar system in the auroral region of Northern Scandinavia. In this case the capital and running costs, and overall control of the project, are being shared between Research Councils or similar bodies in Britain, France, Finland, West Germany, Norway and Sweden. After preparatory discussions and technical studies lasting several years it has been decided to base the main technical centre at Tromsø in Norway, and the administrative headquarters at Kiruna in Sweden. The organisation will be governed by a Council made up of representatives from all the parent bodies, and Professor Tor Hagfors from the Technical University of Trondheim has been appointed as the first Director of the facility.

The incoherent scatter technique originated from a proposal by W. E. Gordon in 1958, for building a very large radar system in order to detect weak Thomson scattering from thermal electrons in the ionosphere. Originally conceived as a way of measuring electron density and temperature in the upper ionosphere from the ground, the method has developed into one of the leading diagnostic tools not only for the upper atmospheric plasma but also for thermonuclear plasmas. From experimental work done in the 1960s, it was soon discovered that both electron and positive ion temperatures could be measured independently, and later that plasma drift speeds and fluxes of super-thermal electrons could also be determined. From these direct measurements, which are made as functions of height and time, many other physical parameters can be derived indirectly: among the most important are the electrostatic field strength, the temperature and wind speed of the neutral air, and energy fluxes. In collaboration with other ground-based techniques, or with rocket or satellite-borne instruments, incoherent scatter can give remarkably

complete information about physical processes and conditions in the upper atmosphere. Incoherent scatter does not however supersede satellite experiments, even though some of the parameters measured are the same. For complete understanding, the upper atmosphere must be seen as a three-dimensional time-varying medium: the radar works in height and time, whereas most satellite measurements are expressed as functions of latitude and longitude.

Incoherent scatter radars are complicated and expensive, requiring very high power transmitters, large antennae and sophisticated data processing equipment. Until now, no more than seven stations world-wide have ever been operating at the same time, and most of these have been at middle latitudes. The EISCAT facility will be only the second station in either of the auroral zones, which are the key regions for observing interactions between the ionosphere, the magnetosphere, the neutral atmosphere and the interplanetary plasma, and where many important and poorly understood phenomena occur. The radar should come into operation in 1978 or 1979, and will then be operated intensively for at least a decade, with its time shared between a routine synoptic programme and special experiments by scientists from the participating countries. In Britain, research groups from a number of Universities and from the SRC's Appleton Laboratory are planning to use the facility. The scientific scope for European workers will be enhanced by the proximity of two rocket launching sites, and of many other ground-based instruments, at well established upper atmospheric observatories in adjacent areas

of each of the three Scandinavian countries. A wide variety of other data will thus be available to complement and enhance EISCAT observations.

In contrast to most of the earlier incoherent scatter sites, the EISCAT system will be designed solely for upper atmospheric research. It will be unusual in having two radars on widely different frequencies. One in the VHF band (at 224 MHz) will be a conventional monostatic type, designed to achieve the maximum sensitivity, and to explore the highest and lowest regions. The other will operate in the UHF band (at 933 MHz), and will be a multistatic radar of the type pioneered in France, and also used in Britain, capable of determining the true vector plasma drift velocity. The transmitters for both radars will be at Tromsø: the VHF receiver and one UHF receiver will be co-sited with the transmitters, while remote UHF receiving stations will be built at Kiruna and at Sodankylä, in Finland, respectively 200 and 400 km distant from Tromsø. Both radars will be designed with very flexible modes of transmission modulation, which should allow for a wide variety of experiments to be conducted. Comprehensive on-line and off-line data processing equipment will also be provided to obtain the maximum possible rates of useful information, and to monitor the progress of observations in real time. These features, together with the uniquely favourable geographical position of the site, should allow European scientists to make significant new contributions to an important field of geophysics. □

Bacterial chemotaxis and methionine

from John S. Parkinson

NEARLY a century ago, Engelmann, Pfeffer and others showed that bacteria are attracted or repelled by various chemicals. Today studies on chemotactic behaviour in *Escherichia coli* and other bacteria have begun to reveal molecular mechanisms of sensory transduction and motility in micro-organisms. The central machinery of chemotaxis is still, however, very much a mystery. Several recent reports suggest that the amino acid methionine may provide the biochemical key to understanding chemotactic behaviour in *E. coli*.

The methionine requirement for chemotaxis is a specific and continuous one. *E. coli* mutants that are unable to synthesise methionine become non-chemotactic when deprived of exogenous methionine and chemotactic ability returns upon restoration of this amino



A hundred years ago

THE first general meeting of the Mineralogical Society of Great Britain and Ireland is held to-day at the Scientific Club, Saville Row, at 12 o'clock (noon), when the chair will be taken by Mr. H. C. Sorby, F.R.S. The first ordinary meeting will be held at the same place and time to-morrow, when a paper will be read on the Scottish Rhombohedral Carbonates, by Prof. M. Forster Heddle, M.D.

from *Nature*, 13, February 3, 274; 1876

acid. Since no other amino acids produce such an effect, it cannot be related simply to effects on protein synthesis. In fact, ongoing protein synthesis is not needed for chemotaxis once the machinery has been assembled.

The behavioural manifestation of methionine deprivation is a change in the organism's swimming pattern. Normal swimming in *E. coli* resembles a random walk in three dimensions with occasional turning or tumbling movements serving to reorient the direction of forward motion. During chemotaxis the cell's tumbling machinery is modulated by chemoreceptors that detect changes in attractant or repellent concentration. Whenever the organism happens to swim toward an attractant or away from a repellent, tumbling is suppressed, which causes net movement in the appropriate direction. Methionine-starved cells are non-chemotactic because they are no longer capable of tumbling. Methionine therefore seems to have a major role in the production or regulation of tumbling movements, events central to the chemotactic process.

Springer *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 4640-4644; 1975) have tried to determine, by examining the effects of methionine deprivation on *E. coli* mutants with very high tumbling rates (tumbly mutants), whether methionine was directly involved in the tumbling process. They found that methionine starvation caused several of the mutants to lose tumbling ability, and that the time required for complete loss of tumbling was related to the chemotactic ability of the strains. Tumbly strains with poor chemotactic responses continued tumbling for several hours after removal of methionine from the medium. One mutant, in fact, never lost the ability to tumble.

Springer *et al.* also showed that exhaustion of the organism's internal reserve of methionine available for tumbling was accelerated by incubating the bacteria in attractant compounds during methionine starvation. Although unchanging attractant concentrations do not ordinarily modify tumbling behaviour, they evidently elicit increased consumption of methionine by the chemotaxis machinery.

Until more is known about the physiological defects of tumbly mutants, it is not possible, on the strength of present evidence, to conclude that methionine is directly used in generating tumbles. The data of Springer *et al.* and results of Aswad and Koshland on *Salmonella typhimurium* (*J. Bact.*, **119**, 640-645; 1974) are perhaps best explained by postulating an indirect role for methionine in the tumbling process. For example, methionine might be consumed in destroy-

ing tumble-inhibiting signals produced by the chemoreceptors. In the absence of methionine these signals would build up (faster in the presence of attractants) until tumbling was suppressed. Tumbly mutants might be less sensitive to such signals, necessitating higher signal levels (hence longer starvation times) to turn off the tumbling machinery in these strains.

A more direct way of investigating the role of methionine in chemotaxis is to determine the chemical fate of exogenously supplied methionine. Earlier work by Armstrong and by Aswad and Koshland had suggested that a compound derived from methionine called SAM (S-adenosylmethionine) was involved in chemotaxis. Since SAM is a donor of methyl groups in many biochemical reactions in the cell, Kort, Goy, Larsen and Adler (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 3939-3943; 1975) reasoned that perhaps the methyl group of methionine is transferred by way of SAM to one or more components of the chemotaxis machinery. To show this, they looked for transfer of radioactive label from the methyl group in methionine to proteins of the cytoplasmic membrane in the absence of *de novo* protein synthesis. Their search focused on membrane proteins because most of the known chemotaxis machinery is intimately associated with the cell's inner membrane.

Kort *et al.* were able to detect a membrane protein that is extensively methylated by methionine. Chemotactic stimuli increase both the rate of methionine utilisation and the amount of label transferred to the membrane protein. Also, the methyl groups on the membrane protein seem to turn over, perhaps reflecting the continuous requirement for methionine in chemotaxis. Chemotaxis mutants, including tumbly and non-tumbling strains, often had very different labelling patterns compared with the wild type. Most of the mutants (defining several different genes) had significantly less activity and correction of these mutations by reversion also resulted in normal methylation levels.

So the methylation reaction seems to be involved in chemotaxis and might conceivably be the primary site of action of methionine in the chemotactic process. But Kort *et al.* found some chemotaxis mutants (in the same genes as those mentioned above) which had normal or greater than normal methylation levels and yet were completely non-chemotactic. Detailed information about methylation and demethylation rates in these mutants should clarify the presently confused relationship between methylation and chemotactic ability.

Even if the role of methionine in bacterial chemotaxis proves to be a minor one, which seems unlikely, it still offers a promising new approach to learning more about the biochemistry of the tumbling process. Future studies will undoubtedly attempt to identify the gene(s) encoding the methyl-accepting membrane protein and to determine their role in chemotaxis through a combination of genetic and biochemical methods. When this has been accomplished, the significance of methionine to chemotaxis will be considerably clearer. □

Heated discussions on Sun and weather

from John Gribbin

1976 seems destined to be the year of the Solar-Terrestrial Relationship conference. International jamborees on the topic are planned later in the year for Frascati (under the auspices of the European Space Agency) and Boulder, Colorado (organised by the American Geophysical Union); a more limited view of these relationships, within the confines of their relevance to meteorology and climate, was the theme of the January 21 meeting of the Royal Meteorological Society, where although the staunch believers in direct solar effects on the weather received a rough ride from some meteorologists there was a firm but reluctant undertone of belief even among the critics that there is evidence of some effect of solar activity on weather.

THE highlight of the meeting was a confrontation between J. W. King (Appleton Laboratory), a firm believer in solar cycle influences on the weather, and B. J. Mason, the Director General of the UK Meteorological Office, the most senior 'establishment' opponent of these ideas. King presented a breathtaking array of data in the form of graphs, charts and correlations between meteorological parameters such as the height of the 500 mbar level and solar parameters, especially the 22 yr cycle which, in magnetic terms, is a more fundamental feature than the superficial 11 yr cycle derived simply by counting sunspots. Among these correlations—in phase as well as in magnitude—most of the figures quoted by King suggested a probability of at least 99% that the relationships are genuine, and he tended to dismiss probabilities of around 95% as rather poor.

Mason responded at length to King's points, complaining that he had presented too many different correlations for the audience to grasp at one sitting, pointing out that they are all *post hoc* deductions and that sunspot cycle fanatics have now had "a hundred years of failure" in attempting to predict weather changes. He stated once again his unwillingness to accept such statistical evidence in the absence of a plausible physical mechanism behind it.

Asked how he had selected the data presented, King replied that they are typical of results obtained by comparing solar activity with published meteorological data, and that in round terms the Appleton team find in 95% of all the published data they have examined a correlation which could arise only about 2% of the time by chance. Mason objected that this meant the data were not new, but when pressed by King to say whether meteorologists should throw out the evidence replied "No", agreeing that solar effects must influence the high atmosphere, and asking that the theorists concentrate on explaining those effects before looking "downstairs" in the troposphere.

Two members of the Met Office then described different studies in which rigorous statistical analysis had failed to remove every trace of a solar influence from their data. B. N. Parker surveyed links between the planetary mean geomagnetic index and surface pressure anomalies on a monthly time-scale over the Northern Hemisphere, and C. K. Folland looked at relationships between sunspot and other quasi-cyclic fluctuations and circulation over the British Isles. The evidence seemed to show significant but not dominant solar influences in some stretches of data at least—not a good enough relationship for forecasting purposes, but suggestive for any theorist studying interactions between the Sun and the Earth. Again, the 22 yr cycle reared its head, and meteorologists took some persuading that this is indeed the more basic solar cycle, not just a harmonic of the 11 yr sunspot number cycle. All this, unfortunately, kept the emphasis of the meeting well away from the short term effects of specific solar events on the atmosphere of the Earth, which surely provide a better handle to grasp the physics of what is going on than any number crunching of cycles, quasi-cycles and pseudo-cycles.

It was left to D. M. Willis (Appleton Laboratory) to summarise possible mechanisms. It might have come as a surprise to meteorologists sceptical of some of King's statistics to learn how much room there is to doubt astrophysical ideas about the Sun and the constancy of the solar parameter. Among many speculations aired before, Willis

rightly emphasised the newer ideas of the influence of solar proton events on the atmosphere. This work is closely related to the study by Reid *et al.* (*Nature*, **259**, 177; 1976) of faunal extinctions at times of magnetic reversals, and suggests that solar cycle influences on the weather may be the accumulation of solar proton effects on the ozone layer. It is also clear, however, that the state of the art in measuring the solar parameter is not yet good enough to rule out the possibility of a change by $\pm 1\%$ over the solar cycle, changing mean global surface temperatures by ± 1 K.

The overall flavour of the meeting seems clear. Solar-Terrestrial relationships affecting the weather are real, but not dominant, and are not well enough understood for their use in Met Office forecasts as yet. But they may provide clues of great value to astronomers investigating the nature of the Sun and solar wind. This explains, perhaps, the apparent rift between meteorologists and space physicists; in fact, they are essentially in agreement on the reality of the link, but view it from very different positions. □

Let booming sands boom

from Peter J. Smith

HARDLY surprisingly in view of their weird effects, sounding sands are incorporated into folklore and legends going back at least 1,500 years. Not that our ancestors were always listening to the same sounds. The most common of the musical sediments is probably squeaking (otherwise known as singing, barking or whistling) sand which produces a high frequency note in the range 500–2,500 Hz. But there is also screeching sand which emits an even higher frequency note (>2,500 Hz) reminiscent of that produced by rubbing a finger around the top of a wine glass; and at the other end of the scale, low frequency booming sand can give rise to a sound like thunder which under ideal conditions can be heard up to 10 km from its source. The essential condition for the production of sound is either natural or forced movement; still sand is inevitably silent sand.

A recent paper by Criswell *et al.* (*J. geophys. Res.*, **80**, 4963; 1975) investigates booming sand, which generates not only acoustic but also seismic waves. Both emissions are produced by natural slumping of the sand,

although they may also be produced manually in short bursts by digging in the sand, by forcing it downhill or even by walking on it. The natural slumping of an area of many square metres of sand may persist up to 15 min, giving rise to a 'roar', or on occasions a 'hum', rather like the sound of a low-flying propeller aircraft. But whatever the sound, it is always accompanied by ground vibrations which can be felt through the feet of anyone standing on or near a booming dune and through the fingers of someone generating artificial booming by digging in the sand. One observer has compared such vibrations to a mild electric shock from household current (50–60 Hz).

Though not particularly common, booming dunes have been reported at more than 30 sites in all continents except Antarctica and, curiously, Australia. The sound frequencies at some of these sites have been estimated before by reference to pitch pipes; but Criswell *et al.* have now carried out the first simultaneous measurement of acoustic and seismic spectra from a booming dune (Sand Mountain in Nevada) using, respectively, air microphones and geophones. The emissions were generated artificially by digging holes in the sand with a flat-bladed shovel.

Not entirely unexpectedly, the scientific aspects of booming dunes turn out to be rather dull and hardly a match for the subterranean ghosts, the shoeing of horses in underground caverns and the clanging of bells in buried monasteries to which sand sounds have been attributed in the past. The most "significant finding" is that the frequency spectrum of a short (<2 s) forced booming event comprises sharp peaks largely in the range 50–80 Hz for both acoustic and seismic emissions. These results throw little light on the mechanism by which the vibrations are generated, which remains unclear; nor do they do much to justify the original aim of the work, which was to see whether a similar mechanism on the Moon could account for surface moonquakes.

Criswell and his colleagues do obtain one interesting result, however. A comparison of an acoustic amplitude trace from a Sand Mountain note with the trace of an 88-Hz organ tone from the opening stanza of Bach's C-Minor Passacaglia and Fugue shows that sand and expertly crafted organ pipes produce notes of comparable purity.

Perhaps Criswell and others should take the hint from Walt Whitman who, tiring of the learn'd astronomer's proofs, figures and charts, "wander'd off . . . in the mystical moist night-air, and from time to time, look'd up . . . at the stars." □

articles

Receptor-binding region of insulin

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X-ray analysis, circular dichroism, receptor binding and biological potencies of chemically modified insulins suggest that the conformation of the insulin molecule is critical to the formation of both the zinc insulin hexamer and the insulin-receptor complex. Results are consistent with an insulin receptor-binding region including many of the hydrophobic residues important to dimerisation in addition to more polar surface residues. There is a further possibility of formation of an antiparallel sheet structure between the insulin and receptor molecules in the complex similar to that between monomers in the insulin dimer.

THE use of synthetic insulin analogues in the treatment of diabetes may become vitally important in the near future. Progress in presently underdeveloped countries means that demand for insulin is likely to exceed supply and this makes the synthesis of analogues a medical priority.

An understanding of the nature of the receptor-binding region of a protein hormone, and especially as complex a molecule as insulin, is essential for the proper design of such synthetic analogues. Fortunately the elucidation of the molecular structure of porcine insulin by X-ray analysis has made possible a more rational approach to the definition of the receptor-binding region^{1,2}. Studies of the biological activities and sequences of insulins from different animals strongly suggest that a largely invariant region on the surface of the insulin monomer is a good candidate for this receptor-binding region. The three-dimensional structure shows that this region might include both A chain residues—A1 Gly, A5 Gln, A19 Tyr and A21 Asn—and adjacent B chain residues—B24 Phe, B25 Phe, B26 Tyr, B12 Val and B16 Tyr (for reviews see refs 3 and 4). Their involvement in receptor binding must now be tested by studying the biological properties of insulins specifically modified at one of these residues.

Modification of insulin may change the biological activity either by reducing the affinity of the hormone for the receptor or by decreasing the ability of the complex, when formed, to elicit a biological response. Either or both these effects may be brought about by direct interference of the modifying group, by changes of charge distribution or by induced conformational changes in the hormone. Thus the position of the modifying

group and the conformational changes induced by it must be defined in detail; this has not been done in previous work. Further, measurements of receptor binding as well as biological activity are essential to test any hypothesis concerning the location of the receptor-binding region, and the effect of chemical modification⁵.

We have carried out parallel studies of high resolution X-ray diffraction, circular dichroism, receptor binding and biological potency in fat cells of native insulin and various chemically modified insulins. The studies have further clarified the factors leading to decreased potency and provide evidence as to the nature of the receptor-binding region.

Chemical modification and X-ray analysis

We studied a series of insulins with modifying groups of increasing size attached to the α -amino group of A1 glycine, one of the invariant residues in the putative receptor-binding region. Techniques of specific chemical modification, developed in our laboratories, were used to prepare A1-acetyl insulin^{6,7}, A1-*t*-butoxycarbonyl insulin⁸ (A1-Boc-insulin) (this has also been prepared by Levy⁹) and A1-2-dimethyl-3-formyl-L-thiazolidine-4-carbonyl insulin (A1-'thiazolidine' insulin)¹⁰. A1-Boc-insulin was in part provided by H.-J. Friesen, Aachen (Diplom. Arbeit, T. H. Aachen, 1975).

Rhombohedral crystals of bovine derivatives formed more easily than those of porcine derivatives. They crystallised at pH 5.8 as opposed to pH 6.2 for native porcine insulin, whereas large crystals of native bovine insulin were prepared only at pH 6.7. For this reason we have studied modified bovine insulins, and also carried out control experiments on native bovine insulin.

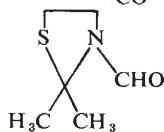
Crystals of the chemically modified and bovine insulins proved to be closely isomorphous with porcine insulin although there are small changes in cell dimensions as indicated in Table 1. X-ray photographs of the A1-'thiazolidine', A1-Boc and A1-acetyl-insulins resemble each other, but the extent of the intensity changes from native insulin increases in the order A1-acetyl < A1-Boc < A1-'thiazolidine' insulin.

Three-dimensional X-ray data for the isomorphous bovine, A1-Boc and A1-'thiazolidine' insulins were collected on a four-circle diffractometer to the resolutions given in Table 1. As the differences between the A1-acetyl and bovine insulins were small, but rather similar in nature to the other two derivatives, we decided that it was not useful to carry out a full high resolution analysis of this derivative.

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Table 1 Comparison of insulins

Insulin	Modifying group	Cell dimensions (Å)		Data resolution (Å)
		<i>a</i> = <i>b</i>	<i>c</i>	
Porcine	—	82.5	34.0	1.9
Bovine	—	82.5	33.8	2.3
Al-acetyl	CH ₃ -CO-	82.5	33.8	—
Al- <i>t</i> -Boc	(CH ₃) ₃ C-O-CO-	82.5	33.5	3.0
Al-'thiazolidine'	-CO-	82.5	33.8	2.3



Structural differences between the insulins were investigated using a series of electron density maps computed with coefficients

$$m(nF_D - (n-1)F_P) \exp i\alpha_P$$

where F_P , m and α_P are the observed structure factor amplitudes, the figure of merit and the best phase for the porcine insulin crystals, and F_D is the observed structure factor amplitude of the derivative crystals. Values of m , α_P and F_P for porcine insulin crystals were provided by Professor Dorothy Hodgkin and her colleagues. Conventional difference maps, with coefficients $(F_D - F_P)$ were used to indicate changes of electron density between the porcine and derivative crystals. But electron density maps calculated with $n = 2$ were used in building models of the derivative structure as the map should give a reasonable approximation of the derivative crystal electron

density and can be compared directly with a model if an optical device is used.

These electron density maps indicate that the bovine insulin dimer is closely similar to the porcine dimer, although there are small changes in the position of one zinc atom and indications of some further cation binding and of a small shift of the molecule in the unit cell. Negative peaks in the difference maps correspond to the sequence differences: A8 Thr and A10 Ile in porcine insulin are replaced by A8 Ala and A10 Val in bovine insulin and these changes are thought to be responsible for the slight differences in packing observed in the bovine and porcine structures. Similar differences of packing occur in the Al-Boc and Al-'thiazolidine' bovine insulins; in addition in both derivatives the region around A1 shows extensive conformational changes in both the molecules of the crystallographic dimer. The changes are qualitatively similar in the two derivatives but are greater in magnitude in Al-'thiazolidine' insulin.

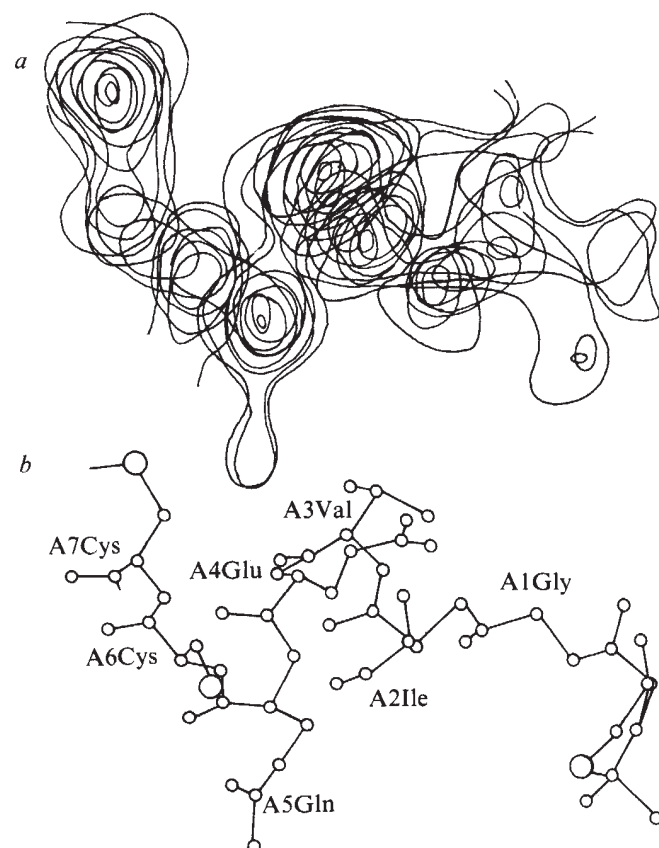
Figure 1 shows the electron density and molecular structure close to A1 in molecule (2) of Al-'thiazolidine' insulin. The thiazolidine group is extended into the solvent region, apparently displacing water molecules, and comprises a distorted extension of the A-chain helix. The Boc group occupies a similar position in Al-Boc insulin. The electron density of the 'thiazolidine' and Boc insulins indicates a rotation of the helix A2-A5 around its axis in a clockwise sense when viewed from the N-terminus. The side chains of A2 Ile, A4 Glu and A5 Gln have been pushed away from the A1 position and the side chain of A19 Tyr has shifted about 1.5 Å from A2. A similar rotation and displacement of side chain groups occurs in the other molecule (1) of the dimer, but the modifying group appears to be closer to the helix axis and the shift of A5 side chain is less. There is also a very small displacement of the residues B24 to B28 away from the modifying groups at A1.

Circular dichroism

Spectra of chemically modified insulins have been published^{7,12-14} but interpretation is difficult since chemical modification usually affects quaternary structure¹⁵. We have shown elsewhere that changes in circular dichroism resulting from changes in insulin concentration and the zinc-insulin ratio can be accounted for by changes of environment of the chromophores on changing the equilibrium populations of monomers, dimers and zinc hexamers. We concluded that the structure of the protomer seen in the crystals of zinc insulin hexamers is largely retained by the molecule in solution. Thus chemical modification of insulin may affect the circular dichroism by changing the population of associated molecules, the overall tertiary structure of the modified insulin being essentially the same as the native molecule.

We have measured the circular dichroism spectra using a Cary 61CD spectrometer, in both the near and far ultraviolet in 0.025 M Tris-HCl buffer pH 7.79, for the Al-'thiazolidine' and Al-Boc insulins in similar concentration ranges and in the presence of zinc ions. Representative spectra are shown in Figs 2 and 3. The spectra of the derivatives at a given concentration correspond generally to the spectra of the native protein

Fig. 1 View of (a) electron density and (b) molecular structure for the A-chain helix and neighbouring residues of Al-'thiazolidine' insulin; the projection corresponds to a depth of 10 Å through the structure of the $n = 2$ map obtained for the derivative (see text).



at higher dilutions, indicating that these derivatives probably associate less strongly than native bovine insulin. A similar comparison of the spectra of the two derivatives suggests that A1-'thiazolidine' insulin associates less than the A1-Boc insulin. This decrease in association can be explained by the change of charge at A1 and small conformational changes induced in the B chain residues B24-B28 which are directly involved in dimerisation. We have already observed that such changes occur in the electron density map but it is probable that the differences between native and derivative structures are slightly greater in the isolated monomer, being decreased in the dimer when the β -sheet is formed. The need to readjust the structure of the modified insulins slightly on dimerisation would explain the decreased ability to dimerise, and account for the decreased dimerisation of A1-'thiazolidine' insulin compared with A1-Boc insulin, a difference which cannot be explained by changed charge or direct interference of the modifying group.

Apart from the shifted concentration and zinc dependence of the circular dichroism, the spectra are very similar, indicating that the derivative insulins also have structures in solution comparable with bovine insulin. There are, however, small differences in ellipticity in both near and far ultraviolet between bovine and derivative insulins at all states of association which may arise from the movement of the A19 tyrosine chromophore as observed in the crystal structure. The general structure of the derivative insulin molecules in solution, however, is probably very similar to that in the crystal.

Biological potency

The potency has been assessed by incorporation of 3-³H-glucose into fat cell lipids^{16,17}. The relative potency of A1-acetyl insulin has been determined as 35-40% (refs 7 and 18). The derivatives exhibit the same maximal activity (efficacy) as insulin with respect to lipogenesis from glucose as has been found for several modified insulins¹⁸. In the experiment shown in Fig. 4a, (left) A1-Boc insulin exhibited a relative potency of 15.6%. The mean of five such experiments was 17.0 ± 3.1 (s.d.). Figure 4a (right) shows a similar experiment with A1-'thiazolidine'

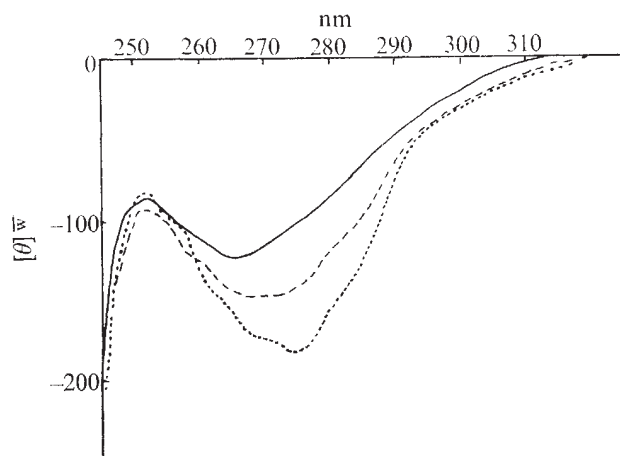


Fig. 2 Near ultraviolet circular dichroism spectra of zinc-free A1-*t*-Boc insulin at 10^{-6} M (—), 10^{-5} M (---) and 10^{-4} M (....), showing the dependence of the spectrum on protein concentration. A similar concentration dependence is shown by bovine insulin. $\bar{w}$, Mean residue weight.

insulin and the mean of five experiments was 12.8 ± 1.6 (s.d.). It is further seen from Fig 4a that A1-'thiazolidine', when added to native insulin in the concentration ratio 10:1, and A1-Boc insulin (ratio 5:1) had additive effects in submaximal doses. This is characteristic of a full agonist which is acting through the same mechanism and with the same rate-determining step as native insulin.

Receptor binding of the insulin derivatives was determined as their ability to inhibit binding of ¹²⁵I-insulin to fat cells as previously described¹⁶. Figure 4b shows that the relative binding affinities of A1-Boc insulin and A1-'thiazolidine' insulin are both similar to the relative potencies. This means that the ratio between the concentration which causes half-

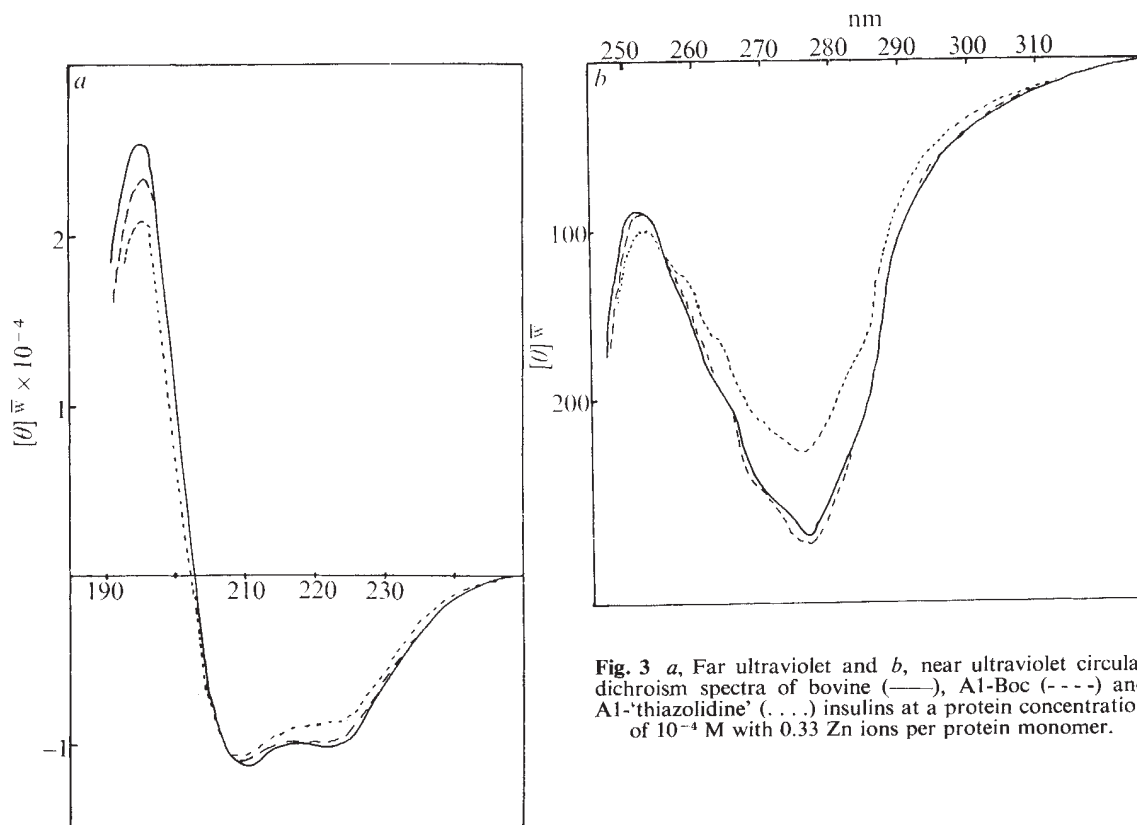


Fig. 3 a, Far ultraviolet and b, near ultraviolet circular dichroism spectra of bovine (—), A1-Boc (---) and A1-'thiazolidine' (....) insulins at a protein concentration of 10^{-4} M with 0.33 Zn ions per protein monomer.

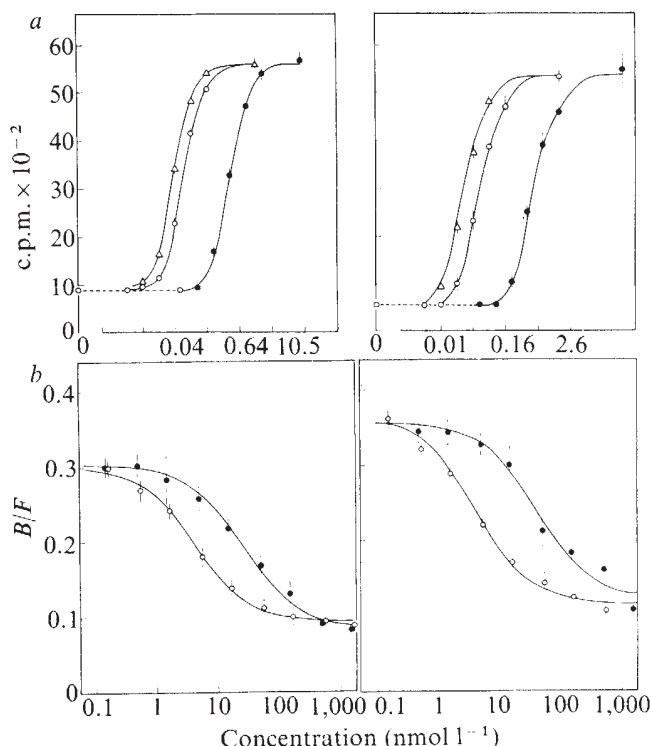


Fig. 4 *a*, Potency of A1-*t*-Boc insulin (left) and A1-'thiazolidine' insulin (right). The ordinate denotes the conversion of 3-³H-glucose (0.5 mM, 0.2 mCi mmol⁻¹) to lipids in fat cells after incubation for 2 h at 37 °C. The potency of the derivatives (●) relative to native insulin (○) was calculated as

$$('K_{\text{action}}' \text{ insulin} / 'K_{\text{action}}' \text{ derivative}) \times 100$$

The effect of addition of derivative to native insulin in the concentration ratio of 5:1 for A1-*t*-Boc insulin and 10:1 for A1-'thiazolidine' insulin is shown as Δ . The smooth curve running through these symbols is the expected addition curve for a full agonist with the potency as calculated from the experiment, that is 15.6% for A1-*t*-Boc insulin and 10.8% for A1-'thiazolidine' insulin. Each point represents the mean of four replicates $\pm$ s.d. *b*, Binding affinity of A1-*t*-Boc insulin (left) and A1-'thiazolidine' insulin (right). The ordinate denotes the ratio ¹²⁵I-insulin bound per l of fat cells/¹²⁵I-insulin per l of medium. The ¹²⁵I-insulin was present in a concentration of 0.7 nM and the abscissa indicates the concentration of native insulin (○) or derivative (●). Each point represents the mean of six replicates $\pm$ s.d. The binding affinity of the derivatives relative to native insulin was calculated as

$$(K_i \text{ insulin} / K_i \text{ derivative}) \times 100$$

where K_i (the inhibition constant) was determined as described¹⁶. In the experiments shown the relative binding affinities were (with 95% confidence limits) 14.8% (13.7–16.0) for A1-Boc insulin and 10.2% (9.6–10.9) for A1-'thiazolidine' insulin.

maximal activity (K_{action}) and the concentration which causes half-maximal displacement of specific binding of ¹²⁵I-insulin (K_d) is about 1:50 both for native insulin and for the derivatives. Since the specific binding sites are believed to mediate the stimulation of lipogenesis from glucose¹⁹ it follows that a derivative-receptor complex causes the same effect as an insulin-receptor complex.

Receptor-binding region

In summary, chemical modification at A1 leads to a loss of the free α -amino group which is mainly positively charged in native insulin at physiological pH. This may account to some extent for the reduced biological potency of the modified insulins, in particular A1-acetyl-insulin. X-ray analysis, however, shows that addition of larger modifying groups at A1 results in changes in the structure of the helix A1-A7 and movement of the side

chain of tyrosine A19, which in turn give rise to distortions in the arrangement of the B-chain residues involved in dimerisation. The changes are similar in A1-Boc-insulin and A1-'thiazolidine' insulin, but are more pronounced in the latter. The X-ray precession photos of A1-acetyl-insulin indicate the presence of small but similar changes in this derivative also. These differences of conformation probably contribute to the variation of dimerisation, receptor binding and potency among the modified insulins; direct steric hindrance may also contribute to decreased receptor binding, but X-ray analysis shows that it cannot account for the decreased ability to dimerise.

The results are consistent with a model in which hydrophobic residues such as B24 Phe, B25 Phe, B26 Tyr, B12 Val and B16 Tyr are involved in receptor binding in a manner similar to their involvement in dimerisation. The affinity constant for receptor binding (10^9), however, is much greater than the dimerisation constant (10^5). Therefore, additional interactions must exist. The proposed receptor-binding region is indicated in Fig. 5. It includes residues such as A1 Gly, A19 Tyr and A21 Asn which play no direct part in dimerisation. Their involvement is also consistent with the low receptor binding of pro-insulin^{3,16}. If this is correct, the B25 Phe side chain—in contrast to dimerisation—would be completely buried in the insulin-receptor complex.

Residues A1, A4, A19 and A21 are probably on the periphery of the receptor-binding region. For A1 this is consistent with the results on receptor binding and potency of modified insulins^{5,18}; the chemical nature of the modifying group can sometimes be varied with little change in the reduction of potency. A19 Tyr may be partly buried when insulin is bound to the receptor although the consensus of results on receptor binding of moniodinated insulins indicates that the affinity is not greatly reduced²⁰.

Certain residues outside the region proposed may also come close to the receptor. It is interesting that turkey and chicken insulins which have a histidine at A8 have considerably enhanced receptor binding^{21,22}. A8 lies just outside the putative receptor-

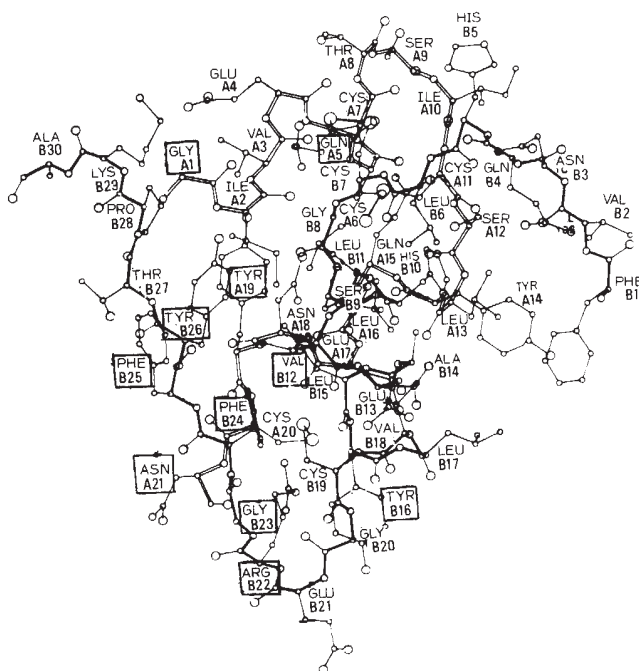


Fig. 5 The residues of the proposed receptor-binding region of insulin are indicated by a box enclosing the residue number. They include those residues on the surface of the molecule involved in dimerisation such as the residues B24–B26, and B12 and B16 in addition to the residues such as A1 and A21 which play no role in the association of insulin molecules. The diagram of the three-dimensional structure of the insulin molecule is taken from ref. 20.

binding region, and variation of this residue between threonine and alanine in porcine and bovine insulin has no effect on receptor binding. It is possible that histidine, which is larger than alanine (bovine) or threonine (porcine), forms further stabilising interactions with receptor residues and so increases the affinity of the receptor for the insulin. But it is also possible that the presence of the histidine induces a small rearrangement in the structure of the receptor-binding residues which increase the affinity. Further structural studies are required to resolve this point.

It is an attractive idea that the receptor binding should in part be analogous to dimerisation, involving a sheet hydrogen bond system between main chain functions as well as hydrophobic interactions of side chains. Hydrogen bonds in an apolar environment would contribute considerably to the stability of the hormone-receptor complex. This emphasises the critical role of the three-dimensional structure in maintaining the arrangement of these groups and would provide a mechanism for attaining a correct relative orientation of insulin and receptor.

The model for insulin receptor binding has three important consequences. First, a change in the three-dimensional structure would decrease receptor binding; this is certainly consistent with these results and with the low receptor binding²³ of des A21 Asn des B30 Ala insulin²⁴ and guinea pig insulin^{22,23,25}, which have very disturbed structures although they retain the B-chain residues thought to bind the receptor. Secondly, full receptor binding is attained by interaction of several insulin residues with the receptor, and loss of any one residue would not be expected to abolish receptor binding; rather it would decrease it as in the case of des-pentapeptide B26-30 insulin, for example^{18,26,27}. Finally, it implies that receptor binding is additive through hydrophobic interactions and formation of specific hydrogen bonds. The importance of hydrophobic interactions may be rather general in hormone receptor binding; certainly it is important in the related hormone glucagon.

This model does not preclude the possibility that certain areas within the receptor-binding region have different roles. It is possible that the interaction of certain residues of the hormone with the receptor contribute little to the stability of the insulin-receptor complex but nevertheless are relatively important to the biological response. This cannot be true of A1 as reduction of potency is paralleled by a reduction of

receptor binding. Further, De Meyts *et al.*²⁸ suggested that the residues involved in intradimer contacts are critical for the phenomenon of negative cooperativity although residues in the region of A1 are not.

As the organisation of the receptor binding region becomes better defined, the intriguing possibility, is that it may soon be possible to mimic this region in an analogue, that is more easily synthesised commercially than is the complex two-chain insulin protein.

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Bursal dissections and gill pouch hormones

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The terrestrial survival of birds and mammals depends on evolutionary changes in cloacal bursae and gill pouches. Comparative dissections of these pouches provide insight into the coordinated functions of cervical endocrine organs, especially as they affect lymphoid tissue.

WHEN the lungs develop during vertebrate embryogenesis, the five paired gill pouches sequentially cease absorbing oxygen to supply epithelia for other cervical organs as follows. The first pouches supply the tympanic cavities and eustachian tubes. The second pouches supply the lateral pharynx and palatine tonsils. The third pouches supply the thymus and inferior parathyroid glands. The fourth pouches supply the superior parathyroid glands, and the fifth pouches supply the ultimobranchial bodies. In each case the

gill pouch endoderm invaginates into the cervical mesenchyme, respectively producing the hollow eustachian tubes, the palatine tonsillar crypts and the stranded epithelium of paired or fused thymus glands, paired parathyroid glands and paired ultimobranchial bodies which fuse with the thyroid primordium derived from the midline endostyle¹.

Bursal dissections

During the embryogenesis of freshwater turtles and birds gill pouch evolution occurs in this way, but in addition, cloacal pouches called cloacal bursae develop. Their structures resemble derivatives of the second to fourth gill pouches.

Dissection of the paired cloacal bursae in freshwater turtles (Fig. 1a and c) reveals pouches opening to the cloaca immediately caudal to the ureteral orifices. The

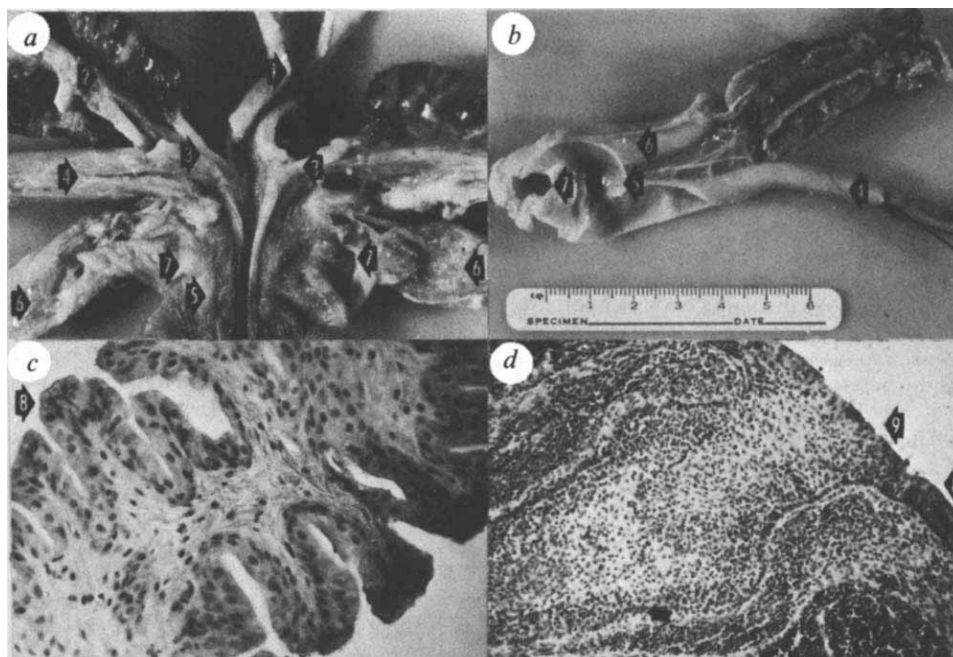


Fig. 1 *a*, Male freshwater turtle (*Cyclemis dentata*). The cloaca (5) is opened in the dorsal midline and attached structures are dissected to expose from above downward: the bladder (1) opening into the cloaca in the midline, the paired kidneys (2) and ureters (3) which open into the cloaca, the colon (4) incised but not opened to the left and the paired cloacal bursae (6) opening into the cloaca caudal to the ureters and the colon at (7). *b*, Pekin duckling with the kidneys to the right and the right ureter coursing over the cloacal bursa of Fabricius (6). The right side of the lower colon (4) and cloaca (5) are resected to expose the opening of the bursa into the cloaca just above the anus and caudal to the ureter (7). *c*, In the turtle bursa the pseudostratified columnar epithelium (8) evaginates into the lumen like gill folds in fish. Submucosal lymphoid tissue is inconspicuous. *d*, In the avian bursa the pseudostratified columnar epithelium (8) invaginates (9) into the submucosa to produce epithelial sheets which are infiltrated with lymphocytes and surrounded by dense lymphoid tissue.

pouches are lined by pseudostratified columnar epithelium similar to that in the gill pouch orifices of fish and tadpoles. The epithelium folds into the underlying connective tissues. Subepithelial lymphoid tissue is sparse.

Dissection of the single cloacal bursa (bursa of Fabricius) in birds (Fig. 1*b* and *d*) reveals a midline pouch open to the cloaca immediately caudal to the ureteral orifices. The pouch is lined by pseudostratified columnar epithelium which folds into the lumen, and invaginates into underlying connective tissue to form bottle-necked epithelial sheets infiltrated with lymphoid cells, surrounded by dense organised lymphoid tissue. This 'lympho-epithelial tissue' resembles the thymus gland of bony fish, tadpoles, turtles, birds and mammals; but differs in that its epithelium does not become separated from that lining the gut lumen and it does not contain Hassall's corpuscles, epithelial cysts, myoid cells or clusters of parathyroid cells (similar to those common in turtle thymus). The avian bursa also resembles the palatine tonsils in that the epithelium invaginates deeply, does not become separate from gut luminal epithelium, is heavily infiltrated with lymphoid cells and is surrounded by dense organised lymphoid tissue. It differs from the palatine tonsils (but not the adenoids) in that the surface epithelium is columnar instead of squamous; and in that germinal centres do not develop in the surrounding lymphoid tissue.

These dissections suggest that the cloacal bursae in freshwater turtles and the avian bursa of Fabricius are homologous structures which, in turn, share homologies with the second to fourth gill pouches.

Bursal functions

In 1590 Hieronymus Fabricius² described the cloacal bursae in birds. In 1733 Perrault³ recorded their presence in some species of turtles. In 1914 Jolly⁴ suggested that such "anal sacs" in turtles are phylogenetic precursors of avian

bursae. Functions of these bursae remained obscure until the 1950s.

In 1958 Smith and James⁵ reported that paired cloacal bursae are rudimentary or lacking in terrapins and sea turtles, but well developed in freshwater turtles which normally hibernate underwater at temperatures less than 40–60 °F. They concluded that freshwater turtles use these bursae as gills when water is flushed in and out of the cloaca during hibernation. Having low basal metabolic rates, especially during hibernation, freshwater turtles (especially Emydidae) derive sufficient dissolved oxygen through cloacal respiration to survive through the winter under ice-covered water^{5–7}. In Pleurodira the cloacal bursae are partially fused⁸ (resembling the single avian bursa). Soft-shelled turtles (for example, *Trionyx triunguis*) lack cloacal bursae, but facultatively respire underwater through the epidermis or through pharyngeal gills⁷ (in the second gill pouch position where mammalian tonsils develop). Retaining functional gills either in the pharynx or in cloacal bursae, the freshwater turtles (like tadpoles) are pivotal in the evolution of respiration and associated adaptations essential to survival on land.

In 1956 Glick *et al.*⁸ reported that surgical bursectomy in newly hatched chicks results in deficient antibody production. Their observation established that the single bursa of Fabricius has an important immunological role in neonatal birds. Moreover, it revolutionised modern concepts of mammalian immunology when the effects of testosterone bursectomy were compared with those of surgical thymectomy in newborn rodents^{9–11}. The new concepts^{11–13}, especially those concerning bursa-dependent B cells, are open to criticism for diverse reasons¹⁴, some of which are as follows. (1) Mammals lack a caudal anatomical counterpart of the avian cloacal bursa^{13,14}. (2) In human babies with "third and fourth pouch syndromes" wherein the thymus and the parathyroid glands failed to develop, in addition to tetany

and various cervico-facial anomalies, variable defects in immunoglobulin production are evident^{15,16}. (3) Surgical bursectomy in chicks on the fourth day of incubation (before the cloaca opens into the chorio-allantoic sac) does not completely suppress immunoglobulin production in surviving chicks, and usually suppresses thymic development¹⁷. Testosterone bursectomy in hatching chicks also suppresses thymic development¹⁷, while testosterone produces thymic involution in mammals¹⁸⁻²⁰. Bursectomy in birds and thymectomy in mammals do not cause serious immunological defects a few weeks after birth^{8,10} once a critical extrathymic lymphoid tissue mass²¹ is established with thymic involution^{14,20} and normal foraging¹⁴.

(4) Among other differences, birds differ from mammals in that they lack tonsils with germinal centres and intestinal Peyer's patches^{4,22}. Birds also lack maternal immunoglobulin passed through the placenta before birth and from milk after hatching. In mammals the tonsillar and Peyer's patch germinal centres commence development with the onset of independent breathing and eating^{14,22}. After development of such germinal centres, endogenous antibody production commences in quantity^{13,23}. Whereas the tonsillar crypts develop such that they may trap newly ingested food along with airborne or food-borne bacteria, the avian bursa is oriented to trap colonic contents containing the undigested or 'commensal' remains. In addition, the avian bursal epithelium demonstrates unique capacities for the pinocytotic digestion of colloidal intestinal contents, while the bursal musculature contracts in synchrony with respiration²⁴. Such distinctive anatomico-physiological arrangements may be cogent to neonatal antibody production in the respective species.

(5) Implantation in Millipore diffusion chambers has shown that thymic and bursal effects on lymphoid tissue development and immunity in mammals and birds are

mediated by hormones produced in associated epithelial cells^{21,26-28}. Although distinctive bursal hormones remain to be isolated, a thymic epithelial hormone (thymosin) with reproducible lymphocytopoietic activity has been partially purified^{29,30}, and corrects immunological and nutritive deficiencies in neonatally thymectomised animals and congenitally athymic human babies³¹. Irrespective of species, many, if not all thymic relationships to body growth, immunity and stress can be explained simply in terms of thymosin's trophic action on DNA metabolism in local and distant lymphoid tissue¹⁴.

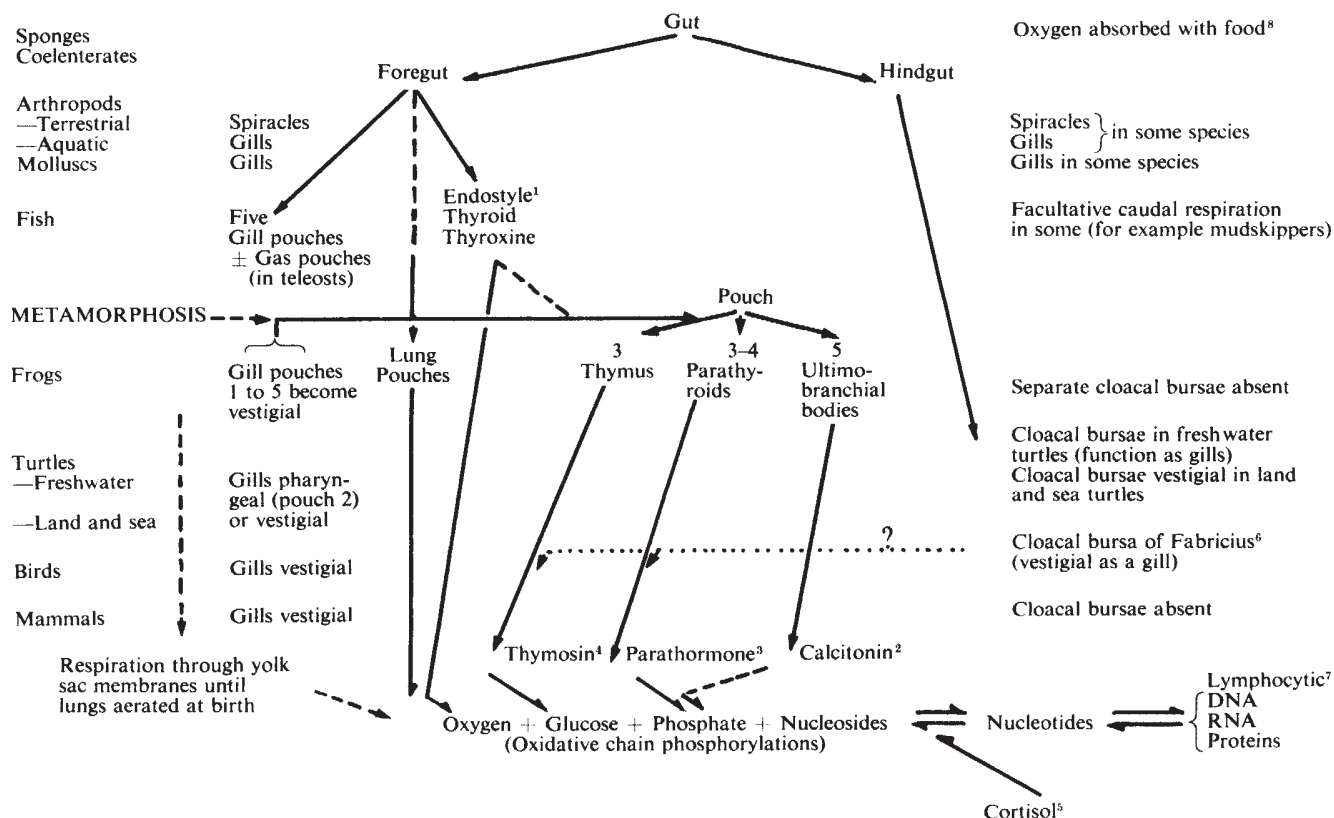
(6) Studies^{32,33} of poikilothermic vertebrates (fish, amphibia, reptiles) reveal evidence of humoral immunity and immunoglobulin production (chiefly IgM) from lymphocytes in thymus, spleen and kidneys before bone marrow develops, and in the absence of cloacal bursae in all species except temperate (as opposed to tropical⁸⁻⁷) freshwater turtles. As Jordan^{22,34}, Marine¹⁹ and Cooper³² pointed out, attention to gill and thymic evolution in anuran amphibians (especially larval frogs) may supply promising avenues of research into the endocrine system.

Thus it can be surmised that in several species of freshwater turtles the cloacal bursae function like gill pouches in fish and tadpoles. In birds which are close phylogenetic relatives of turtles, the cloacal bursa appears to function like an organ hybrid from the second to fourth gill pouch derivatives in mammals, that is the tonsils, thymus and parathyroid glands; and an organ which may contract like gills in synchrony with respiration.

Respiration and cervical endocrine evolution

In embryonic birds and mammals when the third to fifth branchial pouches undergo metamorphosis into cervical endocrine glands and the lung pouches are developing, res-

Fig. 2 Evolution of entodermal pouches concerned with respiration.



piration continues through the vitelline membranes within the liquid environment of the egg or the uterus¹. When breathing under water suddenly changes to lung breathing with hatching or birth, the cervical endocrine epithelia continue to produce hormones which accelerate the internal metabolism of oxygen, glucose and phosphate, especially within lymphoid tissues¹⁴. The teleological relationships are tentatively outlined in Fig. 2.

The superscript numbers in Fig. 2 are interpreted as follows. (1) The thyroid gland, derived from the endostyle¹, produces thyroxine which accelerates metamorphosis^{22,34} and the rate oxygen is consumed to create high energy phosphate bonds in nucleotides³⁵, especially in lymphoid tissues^{14,35}. Thus the rates of DNA synthesis and growth in lymphoid tissues parallel the basal metabolic rate during phylogenesis^{14,36}, ontogenesis^{22,34}, puberty³⁶, alimentation³⁷, and hyperthyroidism^{14,35}, irrespective of the growth rate in other tissues.

(2) The ultimobranchial bodies evolve into the parafollicular cells of the thyroid gland which produce calcitonin³⁵. (3) Parathormone and calcitonin govern phosphate availability and calcium ion concentrations potentially favourable to oxidative chain phosphorylations³⁵. Parathormone stimulates growth of lymphoid tissue, especially in thymus^{18,19}. Also it affects the oxygen affinity of erythrocytes by actions on the phosphates of 2,3-diglycerolphosphate and adenosine diphosphate³⁵.

(4) Thymosin accelerates nucleic acid synthesis, especially in lymphocytes^{29,30} which grow faster, contain higher concentrations of DNA, and release DNA more readily than other cells in the body¹⁴. Because of its insulin-like effect on lymphoid tissue³⁸ and because its action diametrically opposes that of cortisol¹⁴, thymosin's action appears to be on the glucose moiety in oxidative chain phosphorylations. Parallel to thyroxine, it seems to increase the high energy nucleotide-bound phosphate yield per quantity of glucose consumed, particularly in lymphoid tissues. (5) Cortisol interferes with the utilisation of glucose in oxidative chain phosphorylations essential to lymphocytic DNA and protein synthesis³⁹⁻⁴³. Characteristically it engenders lysis rather than synthesis of DNA in lymphoid tissues, especially in the thymus gland^{14,43-46}.

(6) If the "third and fourth pouch syndromes"^{21,16} in immunodeficient human babies are clues, hormones from the avian cloacal bursa seem to have actions like those of thymosin plus variable quantities of parathormone. The combined actions seem to foster RNA synthesis essential to immunoglobulin production, as well as the prerequisite DNA synthesis and formation of appropriate messenger RNA.

(7) Together with lymphotrophic actions, thyroxine, thymosin, parathormone and calcitonin exert others essential to survival in vertebrates destined to crawl out of the water, breathe air and move on land. Actions on bone calcification and erythrocytopoiesis are especially important not only after birth, but also during metamorphosis when sequentially: the thymus, parathyroid and ultimobranchial bodies develop out of the gill pouches¹; the lung pouches commence development¹; the cartilaginous skeleton commences calcification to stiffen the spine and provide a relatively rigid thorax¹; calcifying cartilages are invaded by erythrocytopoietic mesenchyme^{1,22}, while the limbs develop to bear weight; haemoglobin structure changes to carry more oxygen^{12,22,34} organised lymphoid tissues commence to grow in a periarteriole environment of relatively high oxygen tension^{14,47,48}.

With the establishment of intramedullary erythrocytopoiesis and the attendant enucleation of erythrocytes in mammals^{12,22}, lymphocytes multiply around the arterioles in the thymus, spleen, nodes, marrow and intestine to become the most common nucleated cells and constitute 1-3% of the total body mass in the healthy, well fed mammal^{14,36}. Hypoxia engenders sudden lymphocytolysis with shrinkage

of the lymphoid tissue mass in minutes or hours^{14,46-49}. Starvation (eliminating the exogenous supply of glucose, phosphate and precursors for nucleosides) causes lymphocytolysis with shrinkage at a slower rate, but at a rate far exceeding that of cellular attrition in the liver or other tissues^{14,45-50}. The lymphocytolytic effects of anoxia and starvation are mediated by adrenal glucocorticoids^{14,45,46} which make lymphocytic proteins available for gluconeogenesis^{43,46} and release nuclear material rich in high energy nucleotides, especially during stress¹⁴.

(8) The invertebrates and fish are included in the diagram to indicate various forms of respiration which take place during vertebrate phylogenesis⁵¹, as well as mammalian embryogenesis.

If accurate, these teleological correlations based on comparative pouch dissections will necessitate revisions in immunological theory and provide new insights into endocrinology. In turn, when thymosin, parathormone and calcitonin become available in quantity, as are thyroxine and adrenal glucocorticoids, increasingly rational hormonal treatment of diseases involving bones, erythrocytopoietic and lymphocytopoietic tissues may follow.

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letters to nature

Obese 'neutron' stars

WE show here that non-rotating, spherically symmetric, general relativistic 'neutron' stars of mass $\geq 3M_{\odot}$ are consistent with the known laws of physics. In particular, for the 'bag' model of hadrons, we find $M_{\max} \simeq 3M_{\odot}$. The maximum mass for polytropic equations of state is found to be $\simeq 5M_{\odot}$.

This is particularly interesting since it has been repeatedly claimed¹ that the X-ray source Cygnus X-1 is a binary star system containing a black hole accreting matter from its relatively normal companion star HDE226868. The arguments leading to this conclusion, while requiring many assumptions (for example that Cyg X-1 is a binary rather than a triple star system; that the X-ray source is powered by accretion and is not, say, the result of the radiation of spin energy of a rapidly rotating white dwarf; that general relativity is correct beyond its tested domain) depend most crucially on the existence and value of an upper mass limit for 'neutron' stars. (Throughout this discussion the term 'neutron' star is used to refer to a stable matter configuration of mean density $\simeq 10^{14}$ – 10^{15} g cm⁻³, whether the system is predominantly composed of neutrons, quarks, or some other hypothetical state of matter.) Estimates^{2,3} for the mass of Cyg X-1 range from $2.6M_{\odot}$ to as much as $15M_{\odot}$. Most recently, Bahcall⁴ has suggested a value of $M \simeq 4.7 (d/2)^2 M_{\odot}$, where d is the distance to the object in kpc. Since it seems that $d \simeq 2 \pm 0.5$ kpc, one has $M_{\text{Cyg}} \simeq 3$ – $7M_{\odot}$. Based on the claim⁵ that the absolute upper mass limit for non-rotating, general relativistic neutron stars is $3.2M_{\odot}$ whatever the equation of state of matter at supernuclear densities, many authors have concluded that Cyg X-1 must be a black hole. Because of the significance of this result, we have examined

the dependence of the neutron star upper mass the limit on assumed properties of high density matter.

We consider here only the solutions to the hydrostatic equilibrium problem for spherically symmetric, non-rotating, general relativistic neutron stars. The proper gravitational mass $M(r)$ inside a radial distance r is given by

$$M(r) = \int_0^r 4\pi r'^2 \rho(r') dr' \quad (1)$$

where $\rho(r)c^2$ is the total (matter and field) local energy density. The equation of hydrostatic equilibrium relating the pressure $p(r)$ to $\rho(r)$ and $M(r)$, the Oppenheimer-Volkoff equation⁶, is

$$\frac{dp}{dr} = -\frac{GM(r)\rho(r)}{r^2} \left[1 + \frac{p(r)}{\rho(r)c^2} \right] \left[1 + \frac{4\pi r^3 p(r)}{M(r)c^2} \right] \left[1 - \frac{2GM(r)}{rc^2} \right]^{-1} \quad (2)$$

Given an equation of state

$$p = p(\rho) \quad (3)$$

and specific boundary conditions (central density and surface pressure or density) a unique model arises.

For most equations of state $p = p(\rho)$, the hydrostatic equilibrium equation cannot be integrated analytically and so must be evaluated numerically. One simple analytical case, however, demonstrates two of the results we have found numerically. If $p = \alpha \rho c^2$, then a closed form solution for the case of infinite central density is

$$\rho(r) = (c^2/2\pi G)[\alpha/(\alpha^2 + 6\alpha + 1)](1/r^2). \quad (4)$$

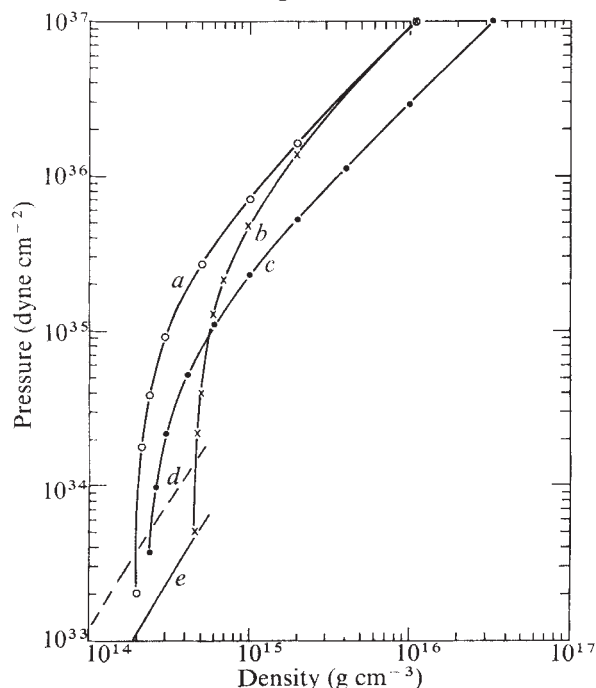
Taking this equation to be valid above some density $\rho_s > \rho_N$ (where $\rho_N = 2 \times 10^{14}$ g cm⁻³ is a characteristic nuclear density) one has for the 'core' of a model neutron star (matched to an outer envelope with a 'realistic' nuclear equation of state, but which in general contains no more than $0.1M_{\odot}$) a mass

$$M = (c^3/G^{3/2})(2/\pi)^{1/2}[\alpha/(\alpha^2 + 6\alpha + 1)]^{3/2}(1/\rho_s^{1/2}) \quad (5)$$

We immediately see that for $p = \alpha \rho c^2$, M is maximised when $\alpha = 1$, that is, for $dp/d\rho = c^2$. We note further that M depends on the inverse square root of that density above which the equation of state $p = \alpha \rho c^2$ applies. We find that for $\alpha = 1$ and $\rho_s = \rho_N$, one has $M_{\text{core}} \simeq 2M_{\odot}$.

The real equation of state of matter at densities $\geq 2 \times 10^{14}$ g cm⁻³ is unknown. Something can, however, be deduced from known properties of several neutron stars. The mass⁷ of the X-ray emitting object Her X-1, probably a neutron star, seems to be $1.3 \pm 0.1M_{\odot}$. The binary star system⁸ containing the pulsar PSR1913+16 has a total mass $2.9M_{\odot}$. If, as seems plausible on evolutionary grounds⁹, the unseen companion is a white dwarf (which must be slowly rotating with a mass $\lesssim 1.4M_{\odot}$), then the pulsar (neutron star) mass is $\gtrsim 1.5M_{\odot}$. Most recently¹⁰ the binary X-ray source Vela X-1, with a pulse period of $\simeq 283$ s, has been found to have a most probable mass of $\gtrsim 1.7M_{\odot}$. Numerically integrating equations (1) and

Fig. 1 Equations of state: *a*, extreme causal equation; $p = (\rho - 2 \times 10^{14})c^2 + P_m$; *b*, equation producing Rhoades-Ruffini result; $p = (\rho - 4.6 \times 10^{14})c^2$; *c*, quark bag model; $p = (\frac{1}{3})(\rho - 2.28 \times 10^{14})c^2$; *d*, free Fermi gas of neutrons; *e*, an extension of Negele's nuclear model.



(2) with the central density as a parameter and assuming that neutrons form a cold, perfect Fermi gas up to arbitrary density, Oppenheimer and Volkoff⁶, and later Misner and Zapolsky¹¹ found the maximum mass to be $\approx 0.7M_{\odot}$. We can now conclude that matter at supernuclear densities, at least in some neutron stars, does not behave as a free Fermi gas of neutrons. Neither does a soft-core potential¹² describe neutrons at high densities, since it yields a value of $M_{\max}$ as low as $0.27M_{\odot}$. Other equations of state, such as that of Malone, Johnson and Bethe¹³ (with $M_{\max} \approx 1.85M_{\odot}$) have upper mass limits only just consistent with the observed masses of neutron stars.

In view of the uncertainties in $p(\rho)$, we have numerically investigated some hypothetical equations of state in order to get a better feel for what $M_{\max}$ depends on. We chose an equation of state to be either a free Fermi gas of neutrons or a more realistic equation¹⁴ of state which accounts for the attractive part of the nuclear potential up to a density ρ_s (the final result is quite insensitive to this choice). For $\rho \geq \rho_s$, polytropic equations of state were chosen of the form

$$p = \beta p^\gamma + p_0 \quad (6)$$

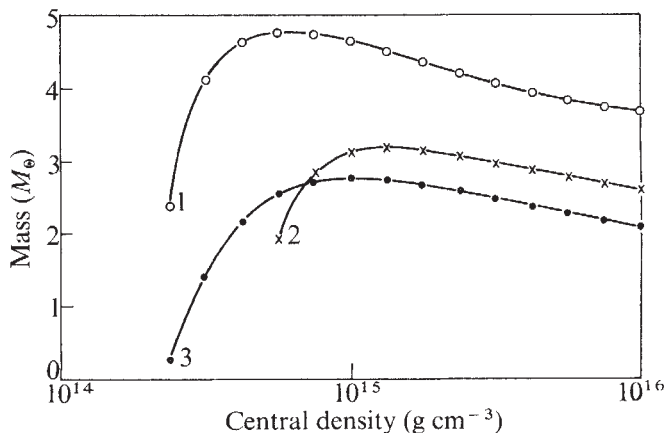
with $1 \leq \gamma \leq 5/2$ and with a range of values of β which includes values for which $dp/d\rho > c^2$. The hydrostatic equilibrium equation was then numerically integrated for given values of β , γ and p_0 , with the central density as a parameter.

The main result of these calculations was that for given values of β and γ (with $p_0 = 0$), $M_{\max}$ varied approximately as $1/\rho_s^{1/2}$, as in the analytical case with $p_{\text{cent}} = \infty$. The greatest value of $M_{\max}$ for $dp/d\rho_{\text{cent}} \leq c^2$ was reached for the case which smoothly matched $p = \rho c^2$ to the 'true' nuclear equation of state at $\rho_s = \rho_N = 2 \times 10^{14} \text{ g cm}^{-3}$, keeping both the pressure and density continuous across the change of state (no 'phase' transition), that is

$$p = (\rho - \rho_N)c^2 + p_M \quad (7)$$

(see Fig. 1, curve *a*). The resulting plot of the mass against the central density is shown in Fig. 2 (curve 1). It can be seen that the curve has a maximum at $\sim 4.8M_{\odot}$. (This agrees with the results for the same model found independently by others^{13,15}.) Such a star is stable and has an equation of state consistent with everything known about nuclear physics at or below nuclear density. The difference between this mass limit and the one found by Rhoades and Ruffini⁵ (curve 2 in Fig. 2) arises almost entirely from the choice of matching density and is just the factor $(\rho_r/\rho_N)^{1/2} \approx 1.5$, where $\rho_r = 4.6 \times 10^{14} \text{ g cm}^{-3}$ is the density to which they extrapolated the equation of state of nuclear matter above nuclear density.

Fig. 2 Mass as a function of central density for: (1) *a* matched to *d*; (2) *b* matched to *d*; and (3) *c* matched to half the free Fermion equation. Stars to the left of the peaks are stable. Matching equations of state *a*, *b* or *c* to either *d* or *e* made little ($< 0.1 M_{\odot}$) difference in the resulting mass limits.



It should also be noted that, for the range of γ we tested the computed values of $M_{\max}$ depended only weakly on γ . But, for such a polytropic equation of state, as $\gamma \rightarrow \infty$, $dp/dr \rightarrow 0$, so that the fluid becomes incompressible. In this limit, for a star of uniform density $\rho = \rho_N$, one finds¹⁵ $M_{\max} \approx 8M_{\odot}$.

These results gain physical significance when one considers the possibility of achieving a very stiff equation of state at or near nuclear density. In particular, the recently proposed 'bag' model of hadrons produces a stiff equation of state at low densities. In essence, for densities just above nuclear, overlapping bags of quarks act as a sea of relativistic massless (or low mass) free fermions. From the results of Degrand *et al.*¹⁶ which fit bag model parameters to elementary particle properties, one finds an equation of state

$$p = \frac{1}{3}(\rho - \rho_B)c^2 \quad (8)$$

where $\rho_B \approx 2.28 \times 10^{14} \text{ g cm}^{-3}$ is one of their two model values of ρ_B . Using this equation of state (curve *c*, Fig. 1), one finds a maximum mass of $M_{\text{bag,max}} \approx 2.8M_{\odot}$ (curve 3, Fig. 2). Most of the mass arises from near the region $\rho \approx \rho_B$: the equation of state for $\rho \gg 10^{15} \text{ g cm}^{-3}$ and for $\rho \ll 10^{14} \text{ g cm}^{-3}$ is virtually irrelevant to the determination of this value. Such a star is, of course, no longer a neutron star but a quark star. (The possibility that supernuclear matter in 'neutron' stars consists of free quarks has also been considered by Collins and Perry¹⁷, though they did not explicitly calculate any stellar models.)

It therefore seems possible to have a stable (not fully collapsed) neutron star with a mass in the range $3\text{--}5M_{\odot}$. These numerical results are consistent with the results of others¹⁸ (for essentially uniform density models) that massive ($\approx 5M_{\odot}$), spherically symmetric, general relativistic neutron stars are in principle possible. Though the overlap between the masses of models considered here and the mass range deduced for Cyg X-1 is small, it seems too early to rule out the possibility that Cyg X-1 is not a black hole but simply a bag of quarks, or that it exists in some other unusual state of supernuclear matter.

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Recognising simulated earthquakes

KOLAR AND PRUVOST¹ have suggested that explosives could be detonated to provide all the characteristics of earthquakes when they are recorded by distant seismographs. Should this experiment be confirmed the effect on efforts to solve the seismological problems which at the moment preclude satisfactory agreement on an underground nuclear test ban would be profound; we present here the results of a relevant experiment.

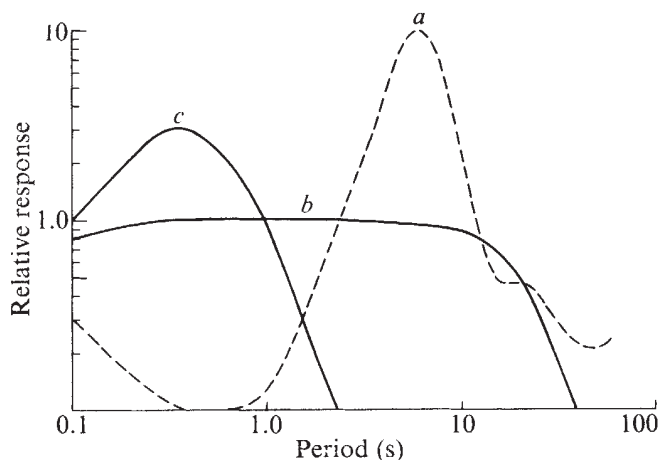
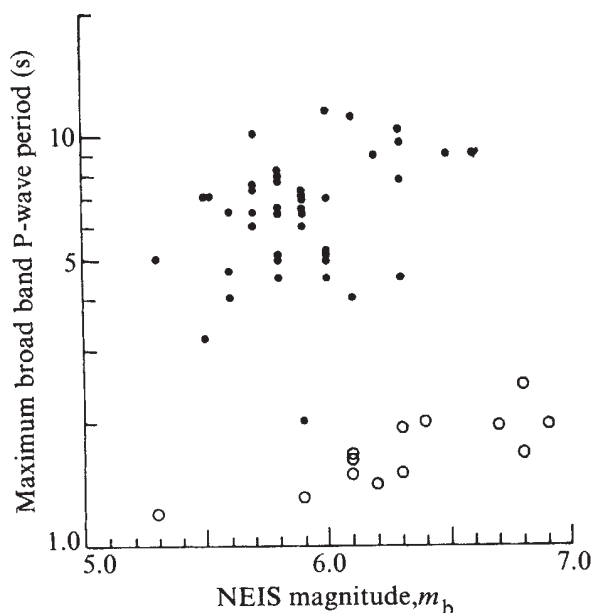


Fig. 1 Typical microseismic noise spectrum (a), (maximum, 10 μ m) superimposed on the broad-band (b) and the LRSM short period (c) seismograph response. The LRSM response peaks here at an amplification of 30,000 but may be operated at amplifications of up to 300,000. The broad band rarely operates at an amplification of more than 10,000.

What Kolar and Pruvost demonstrated was that the direction of first motion could be effectively disguised. They showed that a complex P waveform could be created, and that the conventional magnitudes, m_b and M_s , could be so manipulated that unsuspecting observers would find that all the criteria conformed with those used to establish the occurrence of an average earthquake.

For their demonstration, however, Kolar and Pruvost¹ used recordings made on the narrow band seismograph systems developed especially for the Long Range Seismic Measurement (LRSM) project, primarily used to detect low yield explosions. Bearing in mind Pasechnik's demonstration² of spectral differences between earthquakes and explosions we repeated Kolar and Pruvost's experiment, using a recording from a less sensitive, broader band seismograph (see ref. 3). Our diagram of the relative amplitude responses of the LRSM and broad band seismographs (Fig. 1) include a roughly drawn noise spectrum which illustrates the principal recording problem faced by seismologists. Though

Fig. 2 The maximum broad-band P-wave signal period against the short period magnitude reported by NEIS. ●, Earthquakes; ○, explosions.



the broad-band system includes the peak in the noise spectrum, it falls off at 12 dB an octave beyond a period of 12 s; it is a broad-band instrument only for body waves.

In the first half of our experiment we tested Pasechnik's assertion² that the broad-band body-wave data provide means for distinguishing between earthquakes and explosions. Though the sample is small, the data of Fig. 2 give almost total separation between the P-wave periods of two disturbances above and below the 3-s ordinate. The single exception is known from evidence of a reliable surface reflection pP wave to be an earthquake at a depth of 40 km. The second stage of the experiment consisted of repeating Kolar and Pruvost's procedure for body waves in every detail, except that a broad-band recording of an explosion was used in place of the LRSM record (Fig. 3).

No change of period between the original and mixed signal is observed, so the mixed 'event' remains in the explosion population of Fig. 2. (The experiment was also repeated with Rayleigh waves and, as expected because of the much longer period, the original and mixed signals were virtually identical in shape.) Only the P waves of the larger

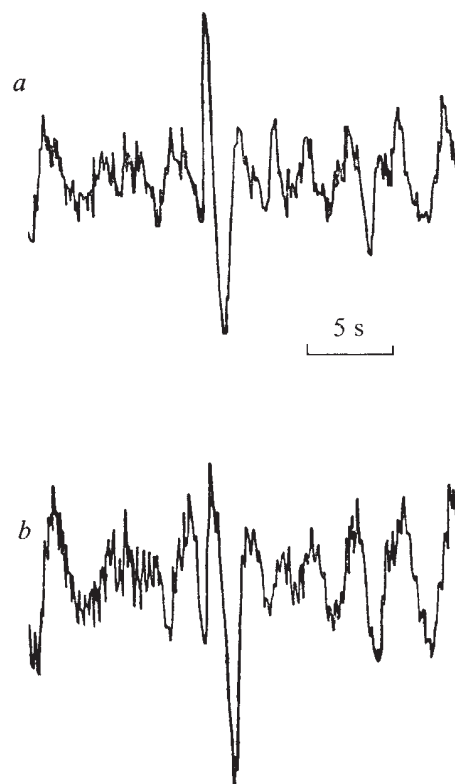


Fig. 3 a, P-wave generated by a Kazakh explosion recorded on the broad-band system and used to simulate the Kolar-Pruvost experiment; b, the resultant seismogram after summation. Note that the predominant period remains almost the same.

explosions would have been observed in this particular case; the earlier ones of the series are below noise level. Therefore, to illustrate the experiment in more detail, to recapitulate the technique, and to carry the test a little further, a synthetic, broad-band explosion signal was summed with no noise (Fig. 4) and the amplitude spectra of the original and final signal were taken (Fig. 5). The peak amplitude remains stable at 2 s; Kolar and Pruvost¹ designed their experiment to reduce the power at 1 s, thereby reducing the apparent body-wave magnitude for their narrow band recording.

The explosion signal shown in Fig. 3a represents a yield of 100 kt on a relatively quiet day in the UK. It implies

that the smallest explosion which could be detected by broad-band seismographs in the UK would be about 50 kt, which is about as far as earthquake simulation technique can be taken, depending as it does on several narrow-band instruments detecting the smaller (by a factor of 10) explosions.

We propose that the broad-band seismograph looks promising as a safeguard against attempts to camouflage moderately large underground tests with a carefully timed series of explosions. It seems likely that the detection level at most stations could be better than 50 kt after array processing (experiments are in hand), though according to Kondorskaya and Aranovich⁴ the resolution of the period discriminant is likely to be weaker at lower magnitudes. Nonetheless a serious would-be violator, in allowing for the possible occurrence of exceptional recording conditions, unknown improvements in techniques and the possible existence of undisclosed stations, would have to credit

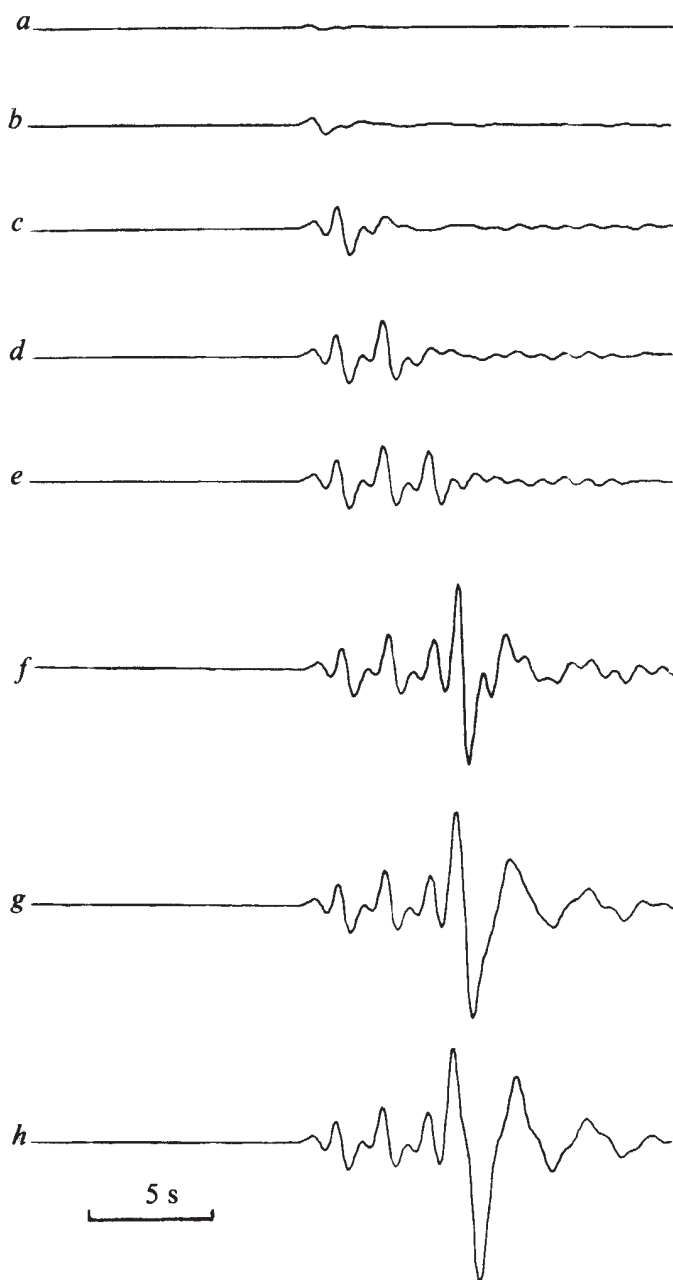


Fig. 4 Model simulation of the multiple explosion, illustrating the development of the final signal. *a*, 3 kt; *b*, + 9 kt, delayed 0.3 s; *c*, + 29 kt, delayed 1.1 s; *d*, + 29 kt, delayed 2.9 s; *e*, + 29 kt, delayed 2.7 s; *f*, + 100 kt, delayed 5.7 s; *g*, + 100 kt, delayed 6.0 s; *h*, + 100 kt, delayed 6.3 s.

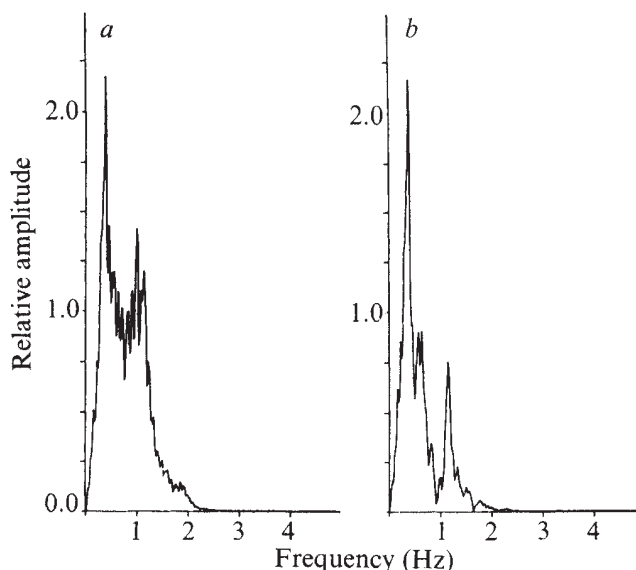


Fig. 5 *a*, Amplitude spectrum of the single explosion used in the simulation experiment; *b*, amplitude spectrum of the resultant multiple explosion. Note that the large, low frequency, spectral peak remains at the same frequency.

'national networks' with somewhat better detection capacities than publicly revealed. A safety factor of two would not be an unreasonable margin to allow. Thus, the geophysical uncertainties operate against the violator.

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Anomalous geomagnetic field during the late Ordovician

MEASUREMENTS of the Lower Palaeozoic magnetic field in the British Isles¹ show that it changed from around $D=355^\circ$, $I=-50^\circ$ to $D=20^\circ$, $I=-54^\circ$ (corresponding to an apparent shift of the south palaeomagnetic pole from 0° W to 35° W along the present Equator) between Ordovician and Devonian times. Following unpublished work by W. E. Tremlett and J. C. Briden, we report here conflicting evidence from the intrusive rocks between Conway and Pwllheli, North Wales. These rocks therefore seem to show either previously unrecognised tectonic rotations or hitherto unsuspected variation in the palaeomagnetic field.

Throughout North Wales, an area of complex Palaeozoic tectonism, a large number of acid igneous intrusions occur whose ages within the Lower Palaeozoic are ill defined. Some bear strong petrogenetic affinities with the Ordovician acid extrusive rocks of the area and on field evidence there is little doubt that such intrusions as the Tan-y-Grisiau microgranite, the Myndd Mawr microgranite, the Penmaenbach rhyolitic intrusion and the Cader Idris granophyre²⁻³ are of Ordovician age, and it has commonly been inferred that the bulk of acid igneous activity in the region is coeval. On the other hand, the view is held by some workers that

Table 1 Fisher statistics of a.f. cleaned remanences of palaeomagnetic sites from Lower Palaeozoic igneous rocks of North Wales

Rock unit	Approximate grid reference	Peak a.f. (mT)	N (sites)	R	k	α_{95}	D*	I*	Palaeomagnetic pole			
									Long.	Lat.	d ψ	d χ
Penmaenmawr granodiorite	SH 700 760	10–40	12	11.6	27	8	164	–71	104E	81S	13	15
Penmaenbach rhyolitic intrusion	SH 750 780	20	12	11.5	21	10	125	–71	112E	59S	15	17
Foel Fras granodiorite	SH 699 695	20	4†	3.9	214	5	207	–78	209E	71S	9	9
Garnfor granodiorite	SH 360 460	20–50	9	8.7	30	13	136	–62	84E	60S	11	14
Carreg-y-Llam granodiorite	SH 340 440	40–80	5	4.9	37	13	126	–71	111E	59S	19	22
Bodeillas granodiorite	SH 320 420	20–60	6	5.8	27	19	120	–55	87E	45S	13	19
Myndd Nefyn granodiorite	SH 320 410	30–80	7	6.5	11	14	139	–36	55E	44S	12	20
Gurn Ddu granodiorite	SH 400 470	20–60	6	5.8	22	9	244	–72	233E	55S	22	25
Yr Eifl microgranite	SH 350 450	20	6†	5.9	62	14	82	–65	122E	32S	15	19
Cader Idris basalts	SH 710 146	20–60	10	9.4	13	14	208	–73	229E	73S	18	22
Overall mean of above results (all sites)			69	63.1	11	5	150	–69	96E	72S	8	9
Overall mean of above results (excluding Myndd Nefyn and Cader Idris)			52	50.0	17	5	143	–71	108E	68S	8	9

*All mean directions quoted are *in situ* except for that of the Cader Idris basalts which has been tilt-adjusted by simple rotation about the present strike.

†Statistics presented are the within-site result from a single site at Foel Fras and Yr Eifl.

The notation used is as follows: R is the resultant of N unit vectors, k ($= N-1/N-R$) is the estimate of Fisher's¹³ precision κ , α_{95} is the semi-angle of the cone of 95% confidence about the mean direction D (declination in degrees east of north) and I (inclination from horizontal, positive indicating downwards), $d\psi$ and $d\chi$ are the semi-angles subtended by the major and minor axes of the ellipse of 95% confidence about the mean pole position.

some intrusions belong to a late stage of the Caledonian orogeny. In particular, W. E. Tremlett has argued that the granodiorite complex of northern Llyn is associated with NE–SW Caledonoid faults of that area, and is therefore probably of early Devonian age; it is geochemically distinct from the Ordovician acid rocks^{7–9}. He has also argued on field evidence that the Penmaenmawr microgranodiorite is post-orogenic, and in disagreement with Davies⁶ assigns a similar age to the Foel Fras granodiorite 6 km south of Penmaenmawr (W. E. Tremlett, personal communication). The Penmaenmawr intrusion has given a K–Ar age of 375 Ma¹⁰ from an admittedly weathered sample. The Llyn granodioritic intrusions have yielded Hercynian ages (R. M. McIntyre, personal communication) but there is no geological evidence to suggest massive igneous activity in North Wales at that time, and these apparent radiometric ages are therefore to be regarded as minimum estimates.

Many of the intrusions have been sampled during our new study and the palaeomagnetism of those possessing stable remanences after a.f. cleaning is reported here (Table 1, Fig. 1). Typically at least six independently orientated specimens were obtained at each site. Total NRM's were generally well grouped, but in a few cases large randomly directed magnetically soft components were removed in low alternating fields, enabling a well grouped stable remanence to be isolated. The criterion for optimum magnetic cleaning is that of Briden¹⁴. In all cases, progressive a.f. demagnetisation experiments have shown the observed remanences to be of high coercivity and in most cases directionally stable to peak alternating fields > 100 mT. Microscopic examination of polished specimens generally shows euhedral-subhedral magnetite grains with lamellar intergrowths of ilmenite, a textural combination associated with high temperature (> 600 °C) petrogenesis¹¹. In spite of the unavailability of baked contact tests or other geological evidence for the age and origin of remanence, the stable NRM is therefore likely to be a TRM acquired during initial cooling. With the exception of the results from the Myndd Nefyn granodiorite, which may be deviant due to slight relative movement in an area of undeniably complex faulting⁹, the *in situ* remanences are well grouped with an overall mean of $D=143^\circ$, $I=-71^\circ$ corresponding to a conventional palaeomagnetic pole at 108°E , 68°S .

This mean pole is nearly 90° away from published Ordovician–Devonian poles for Britain and an explanation must be sought.

Inevitably there are time gaps in the palaeomagnetic record, and it may well be that the new data fall in one of these. Gaps of the order 10^7 yr are to be expected given the sparseness of published data. Therefore if the discrepancy is to be explained in terms of polar shift on the assumption of an axial geocentric dipole field, then apparent polar wander at an average rate approaching $20^\circ \text{ Myr}^{-1}$ has to be invoked. This is an order of magnitude greater than apparent polar wander arising from plate motions in more recent geological times. Even if it were accomplished by minimum lateral motion (given by the palaeolatitude change) together with rotation about a vertical axis close to the sampling area (to accommodate the declination discrepancy), motion of $> 50 \text{ cm yr}^{-1}$ is required, which is still excessive on any plate tectonic analogy.

An interval of 10^7 yr might also be thought reasonable for the emplacement of these widely spaced North Wales bodies. It is hard to imagine an overall intrusion time of $< 10^6$ yr for such a province. Hence the possibility that these rocks record a single geomagnetic polarity transition is effectively excluded because estimates on Tertiary to Recent transition times indicate that they typically take 10^3 – 10^4 yr. Nevertheless it is intriguing to note that the pole position from the North Wales studies is almost equatorial to the typical Ordovician palaeomagnetic field. This is in common with the finding of Shaw¹² that the field paused in this half-way state during a polarity transition recorded in the palaeomagnetism of some Icelandic rocks.

In an area of such complex structural history as North Wales it might be expected that the *in situ* remanences of the intrusions require adjustment for tilt and/or local rotation. This does not, however, seem to be the case, as these massive intrusions, many of which have near vertical 'stock-like' shapes, would require an unlikely 60° northward rotation about a horizontal axis to remove the palaeomagnetic discrepancy. Also, they are seen to carry near vertical contemporaneous dikes. Table 1 and Fig. 1 also contain the tilt-adjusted palaeomagnetic results from the structurally simple Ordovician Upper and Lower Basic Lavas of Cader Idris. Their overall mean magnetisation falls within the group of *in situ* results from the intrusions. The magnetic stability of the lavas was comparatively low, but the similarity of the two results does reinforce the suggestion that the intrusions have suffered little tectonism. Therefore unless evidence is produced to show that the bodies sampled in this study, which are geographically

widely scattered from Cader Idris to Conway, have all been rotated by the same amounts by a bodily movement of North Wales, then their *in situ* remanences must be considered to define the geomagnetic field at the time when those remanences were acquired.

In North Wales there is no geological evidence of any large scale post-Caledonian thermal event at high enough temperatures to explain the stable remanent magnetisations observed in this study. In any case the only known palaeomagnetic field corresponding to these results is that of Tertiary to Recent times. The small amount of Tertiary igneous activity in North Wales could not have been responsible for magnetising rocks on the large scale necessary to explain the observations.

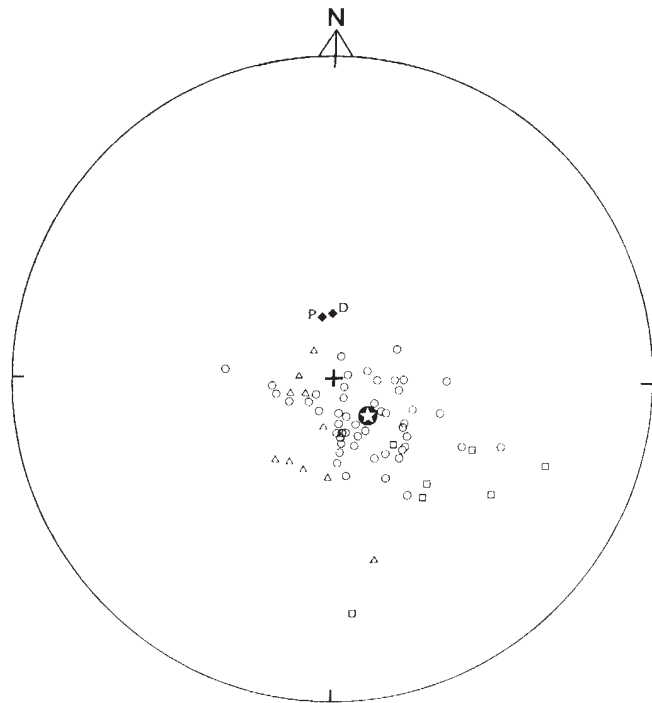


Fig. 1 Stereographic projection of site mean remanences after a.f. cleaning of intrusives from North Wales, and the basic lavas of Cader Idris. ○, *In situ* remanences in all intrusive rocks except Myndd Nefyn; the star indicates their overall mean direction; □, the *in situ* results from Myndd Nefyn; △, tilt-adjusted results from the basic lavas of Cader Idris. The present axial geocentric dipole and the present geomagnetic field directions are marked ♦ and labelled D and P respectively. Open symbols indicate negative (upwards) inclinations and closed symbols positive (downwards) inclinations.

All conventional interpretations of the results of this study are therefore unsatisfactory, and instead, the possibility of an anomalous geomagnetic field lasting several million years during the Lower Palaeozoic must be seriously considered. It is most likely that we have recorded only one such anomalous event in this study because of the improbability of successive intrusions repeatedly occurring at similar geomagnetically anomalous instants. Evidence of precisely when this anomaly might have existed is inconclusive. Its occurrence in late Ordovician times would, however, be compatible with the inferred primary TRM of the intrusions, their possible Ordovician age and the similarity of their remanence directions with that of the proven Ordovician Cader Idris lavas.

The reality of an anomalous magnetic field lasting $\sim 10^7$ yr would be of considerable geomagnetic interest. Whether

it exists or not should be capable of resolution by palaeomagnetic studies elsewhere in the world.

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Palaeomagnetism of a slowly cooled plutonic terrain in western Greenland

PALAEOMAGNETIC results from a high grade Precambrian terrain in western Greenland show many features attributable to magnetisation during very slow cooling caused by gradual uplift and erosion. They may locate a whole segment of the Laurentian apparent polar wander (APW) path with unusually high precision and also indicate the probable direction of movement.

The magnetic effects described here are analogous to those experienced by radiometric systems during slow cooling^{1,2}, and are attributable in part to the fact that magnetic minerals have a range of blocking temperatures at which the magnetisation becomes effectively fixed. The effective blocking temperatures of magnetic grains will be considerably lowered during slow cooling, possibly to as low as 200 °C (refs 4 and 5) so that magnetisation would be acquired during the later stages of unroofing, when moderate erosion rates can be expected. Average present-day erosion rates have been variously estimated at 30 and 76 m Myr⁻¹ (refs 6 and 7, respectively), and if a slightly higher rate of 100 m Myr⁻¹ is assumed together with a geothermal gradient of 20 °C km⁻¹, and a blocking temperature range of 50 °C for stably magnetised grains, then the magnetisation of any one specimen would take 25 Myr. Also, because the magnetisation will be acquired as the rocks pass through the (presumably horizontal) isotherms corresponding to their critical range of blocking temperatures, rocks at higher structural levels will become magnetised earlier than those at lower levels. For example, with an erosion rate of 100 m Myr⁻¹, a difference in level of 2 km would correspond to a difference in the mean time of magnetisation of the order of 20 Myr.

Slow cooling during unroofing should therefore produce several effects in the palaeomagnetic record.

- Apart from the effects of APW all of the rocks formed before uplift should be magnetised in the same direction and give the same pole position, even though geological evidence indicates that they are of different ages.

- Because secular variation should effectively be averaged out within each specimen, between-site scatter attributable to this cause should be small.

- If APW occurred during the magnetisation period then poles from sampling sites at different original structural levels should be strung out along a trend corresponding to that particular section of the APW path, with higher sites at the older part of the trend and lower sites at the younger part.

● If they have the same mean blocking temperature different sampling sites at the same structural level should give poles close together. Any variation in mean blocking temperature, however, will clearly affect the time of magnetisation, and so poles from sites with high blocking temperatures should be displaced towards the older part of the trend compared with sites with lower blocking temperatures at the same level. Because of this it is unlikely that there will be a perfect relationship between the position of a pole in the trend of poles and the structural level of the corresponding sampling site. Determination of mean blocking temperatures in the laboratory should confirm the sense of time along the trend indicated by comparing the position of poles from sites at different structural levels.

● As any specimen has probably taken millions of years to cool through its critical range of blocking temperatures it should contain the magnetic record of that interval of time. Progressive thermal demagnetisation should therefore effectively move the magnetisation of a specimen from the lowest to the highest blocking temperature direction. Two possibilities arise. First, if the blocking temperature spectrum has two peaks corresponding, for example, to haematite and magnetite, then two distinct magnetisations may be present, an older one corresponding to haematite and a younger one corresponding to magnetite. Progressive demagnetisation would cause the magnetisation to move towards the older direction, but the actual movement would simply follow the great circle connecting the two directions. Such results have been reported from the Grenville province^{3,4}. Secondly, if there is a continuous spectrum of blocking temperatures attributable, for example, to a continuous variation in grain size or ionic substitution, then a continuous spread of directions of magnetisation should exist between those corresponding to the highest and lowest blocking temperatures. Progressive demagnetisation would again cause the magnetisation to move towards the older direction, and the actual movement should indicate, after suitable vector analysis, the form of that particular section of the APW path.

● If rocks cool through their critical range of blocking temperatures during a period when the Earth's field is reversing itself at the same rate as at present they would be effectively demagnetised⁵. For rocks to acquire any magnetisation they must cool either during a prolonged period of constant polarity, or in a period when one polarity is dominant. Thus in a slowly cooled terrain one would expect either that the rocks should carry no stable magnetisation, or that they should all be magnetised with the same polarity.

The results reported here are from an area about 20 km square near Itivdleq some 40 km south of Holsteinsborg in western Greenland. The area is just within the early Proterozoic, Nagssugtoqidian Mobile Belt, close to its gradational boundary⁶ with the Archaean craton to the south. The terrain comprises granulite and amphibolite facies gneisses cut by dolerite dykes which vary from fresh to almost completely autometamorphosed but which are essentially undeformed. There is strong geological evidence¹⁰ that the area was at metamorphic temperatures when the dykes were intruded.

Stable magnetisation was found in both dykes (18 sites) and gneisses (4 sites), and after suitable alternating field (a.f.) demagnetisation most sites showed an unusually low within-site scatter of directions. Fourteen sites had a precision parameter, k , of better than 300 (corresponding to a circle of 95% confidence with a radius α_{95} of less than 3.2°), and at no site was k less than 50 ($\alpha_{95} = 7.9^\circ$). Largely because of the accuracy with which the site poles are defined the effects of slow cooling can be recognised.

Figure 1 shows that 18 poles (15 dykes and 3 gneisses) are arranged in a linear trend approximately 10° wide and

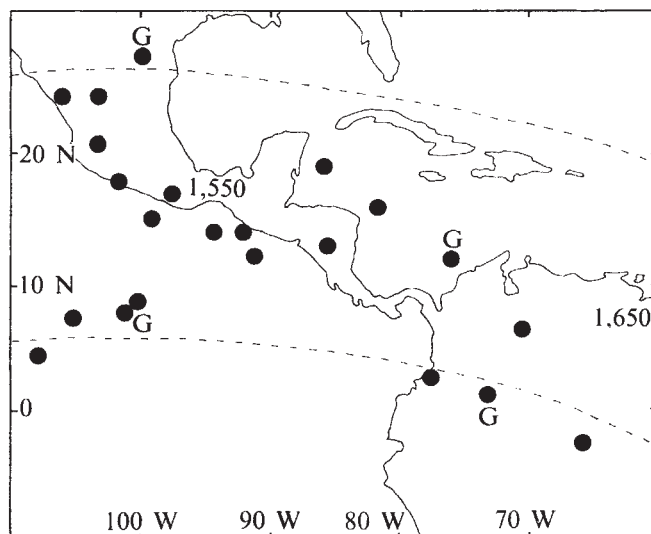


Fig. 1 The 22 site poles. Each is the mean of, generally, eight separately oriented specimens. The 18 dyke poles are unlabelled: G, the four gneiss poles. The poles have been rotated to correct for the drift of Greenland away from North America¹¹ to allow comparison with the appropriate part of the latest published APW path¹² for Laurentia (dashed band, with assigned ages in Myr). Most site poles initially showed southerly movements with progressive a.f. demagnetisation, presumably due to the removal of recently acquired viscous components, and at 7 sites (see text and Fig. 3) these were followed by systematic south-easterly movements. The pole selected for each site was generally either at the end point of the southerly movement or at the point where the southerly movement changed to a south-easterly movement, and was usually at a higher demagnetising field than that which gave the best within-site precision.

45° long, running NW–SE, and making a slight angle with the appropriate portion of the latest Laurentian APW path¹². The remaining 4 poles are grouped together just to the south-west of the trend, which cannot be explained at present. All 174 specimens examined were magnetised with the same polarity. After correcting for the drift of Greenland away from North America¹¹ the mean direction of magnetisation for all 22 sites gives a pole at $13^\circ\text{N } 91^\circ\text{W}$, with $k=47.1$ and α_{95} of 4.6° .

All of the sampling sites were within 50 m of sea level, so if the linear trend of poles is attributable to differences in the original structural level there must have been a slight tilting of the area subsequent to magnetisation. For example, a tilt of 5° , which would be difficult to detect geologically in such a terrain, would be equivalent over a horizontal distance of 20 km to a change in the original level of 1.8 km. This possibility is supported in this case by a general, though imperfect, relationship between the position of a pole in the linear trend of poles and the geographical position of the corresponding sampling site (Fig. 2): most of the sites in the NNW part of the area give poles in the south-eastern part of the trend, and sites in the SSE part of the area give poles in the north-western part of the trend. If the north-western part of the trend is the younger, the results suggest that the SSE part of the area represents the lowest structural level, that is, that there has been tilting of the terrain towards the NNW. The poles from sites D16, D17 and G20 (Fig. 2) clearly do not conform to this pattern; those sites are the most northerly in the area and so their poles should plot at the south-eastern end of the trend. But a prominent E–W fault, for which there is some geological evidence that the movement has been predominantly vertical (D. Nash, personal communication), separates them from the remainder of the sampling area. The apparent displacement of the anomalous poles could thus be caused by the area north of the fault having moved up with respect to the area to the south.

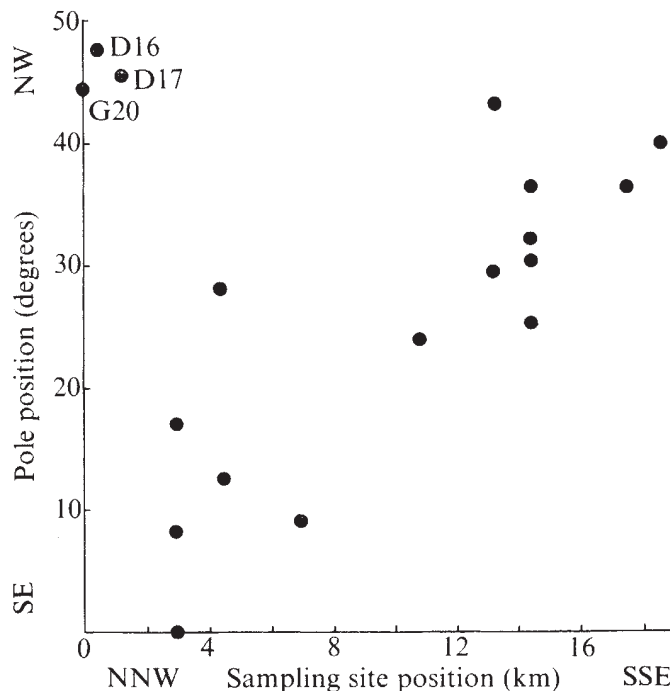


Fig. 2 The relationship between sampling site positions and corresponding pole positions for the 18 poles in the linear trend. Ordinate, the angular distance north-west along the linear trend to each pole from the D13 pole (the most south-easterly pole); abscissa, distance SSE to each sampling site from an imaginary ENE trending line through site G20 (the most northerly site).

If the linear trend of poles is due to APW during slow magnetisation then the scatter of poles attributable to other causes, represented by the 10° width of the trend, is equivalent to an angular standard deviation of about 4° . This is much less than the values of 9 – 24° quoted for secular variation for the appropriate palaeolatitude¹³ indicating either that magnetisation was extremely fast, which seems inconceivable on geological grounds and would not in any case explain the linear trend, or that it was so slow that secular variation was effectively averaged out.

During progressive a.f. demagnetisation the site mean pole at seven sites showed small but very systematic movements always in a south-easterly direction (Fig. 3). As there is probably a reasonably close relationship between coercive force and blocking temperature, the systematic movements may represent the movement of the magnetisation from a younger direction, corresponding to grains with lower blocking temperatures, to an older one attributable to grains with higher blocking temperatures. Vector analysis of the movements indicates that, in general, the component actually removed in each step moves progressively in a south-easterly direction, suggesting either that there are two distinct magnetisations with overlapping coercivity spectra, or that there is a continuous spread of magnetisations. The latter seems more likely because the average direction of systematic movement is close to the trend of the poles, indicating that both record the APW path over that period of time. The linear trend of the poles is not greatly different in direction from the trend of the latest published APW path at that point¹², and the sense of time along the trend of poles as indicated by the systematic movements (younger towards the north-west and older towards the south-east) is in accord with the age dates assigned to the APW path.

Thermal demagnetisation studies were carried out on six specimens from sites which all showed systematic south-easterly movements during a.f. demagnetisation. Up to 520°C the mean pole moved generally southwards but

between 520 and 560°C , when by far the major decrease in the intensity of magnetisation occurred, the mean pole moved systematically in a south-easterly direction very close to the mean direction of the systematic a.f. movements (Fig. 3). It therefore seems that the stable magnetisation of the specimens resides in grains of magnetite or titanomagnetite which have blocking temperatures (measured on the laboratory time scale) of between 520 and 560°C , and that the systematic south-easterly movements also occur over that interval.

According to the assigned ages on the latest APW path the trend of poles covers an age range from about $1,525$ to $1,650$ Myr BP, but an examination of the well dated poles in the area suggests that this portion of the APW path may be about 100 Myr older than indicated. Ages are not yet available from the sampling area, but regional K–Ar mineral ages from the Nagssugtoqidian Belt as a whole range from $1,650$ to $1,790$ Myr BP, and provisional U–Pb studies on zircons give ages of $2,200$ – $2,600$ Myr BP (ref. 14). There thus seems to be good agreement between the time of magnetisation and the setting of the K–Ar mineral clocks, indicating that the magnetisation was probably acquired during a fairly late stage of uplift and cooling long after the peak of metamorphism.

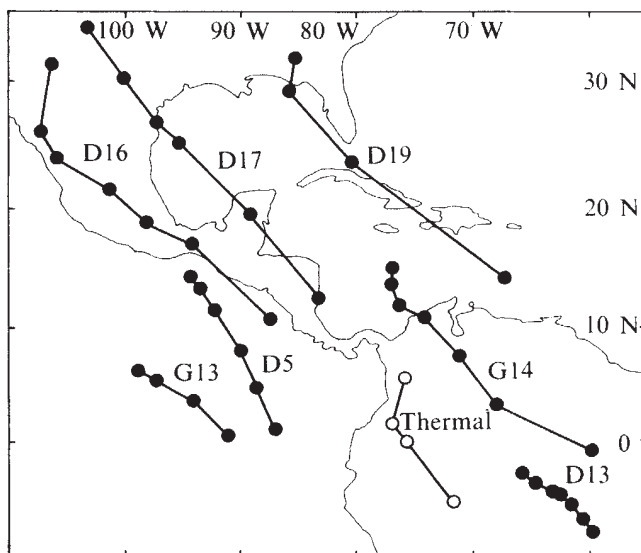


Fig. 3 Systematic south-easterly movements of the mean pole with progressive a.f. demagnetisation at seven sites. For several sites the final part of the southerly movement is also shown to illustrate the transition from southerly to south-easterly movement. ○, The movement of the mean pole of six selected specimens with progressive thermal demagnetisation between 500 and 560°C . All of the poles have been corrected for the drift of Greenland as in Fig. 1. The poles for D17 and D19 have also been moved 10° north, and the thermal poles 9° south-west, to improve the clarity of the diagram. The demagnetising fields (oersted) were: D5: 200, 300, 400, 600, 800, 1,000; D13: 150, 200, 300, 400, 600, 800, 1,000; D16: 0, 50, 100, 200, 300, 400, 600; D17: 50, 100, 150, 200, 400, 600; D19: 100, 150, 200, 400; G13: 600, 800, 1,000, 1,200; G14: 100, 150, 200, 400, 600, 800, 1,000. For thermal demagnetisation the temperatures were 500 , 520 , 540 and 560°C .

The results thus seem to show all of the characteristics to be expected from a slowly cooled terrain. If results of this type prove to be common in plutonic terrains they will not only provide valuable details about apparent polar wander, but could also be a useful tool for investigating movements, such as tilting and faulting, which have occurred subsequent to magnetisation, and which often cannot easily be studied by other methods.

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Effect of depth of burial and tectonic activity on coalification

THE study of variations in coal rank is important to coal geologists and the coal industry, and a proper understanding of the coalification process is also important to oil exploration geologists. The rank of coals, which occur as thin seams or lenses in oilfield sediments, can be regarded as an indicator for the degree of maturation of the organic matter (kerogen), finely dispersed in the sediments. Coal and kerogen are somewhat similar in chemical composition, and so are controlled by the same physical and chemical factors. If tectonic pressure can be shown to advance coalification, then it follows that a higher maturation of kerogen can be expected near a fault zone.

Coalification occurs when original plant material becomes buried, and gradual changes in its chemical composition and physical properties take place. The changes can lead to the formation of lignite, brown coal, bituminous coal or anthracite. During the late nineteenth century, it was widely supposed that burial pressure on coal was responsible for the advancement of coalification. In some tectonically disturbed coalfields, for instance, the presence of high rank coals were found which led geologists to believe that tectonic pressures accelerated coalification¹. Other studies in coalfield geology and coal chemistry have convincingly shown that the role of pressure is insignificant in the process of coalification^{2–6}, and the results of experiments simulating coalification⁶ indicate that the lower the pressure, the more rapid the coalification at a given temperature. From thermodynamics it is known that the gross reaction in chemical processes, including coalification, is determined mainly by temperature and time, and that static pressure retards reactions because it restricts the removal of reaction products³. A coalification pattern which does not seem to fit these studies has been reported for the Bowen Basin of Queensland, Australia (see ref. 7; and Fig. 1). We believe, however, that the pattern in this area is attributable to differences in the original depths of burial below subsequently eroded sediments.

During the Palaeozoic, a sediment-filled, linear downwarp formed along the eastern margin of Australia, separating an old land mass to the west from a rising orogenic belt to the east. During the Permian, coal-bearing formations were deposited; they were followed by as much as 5 km

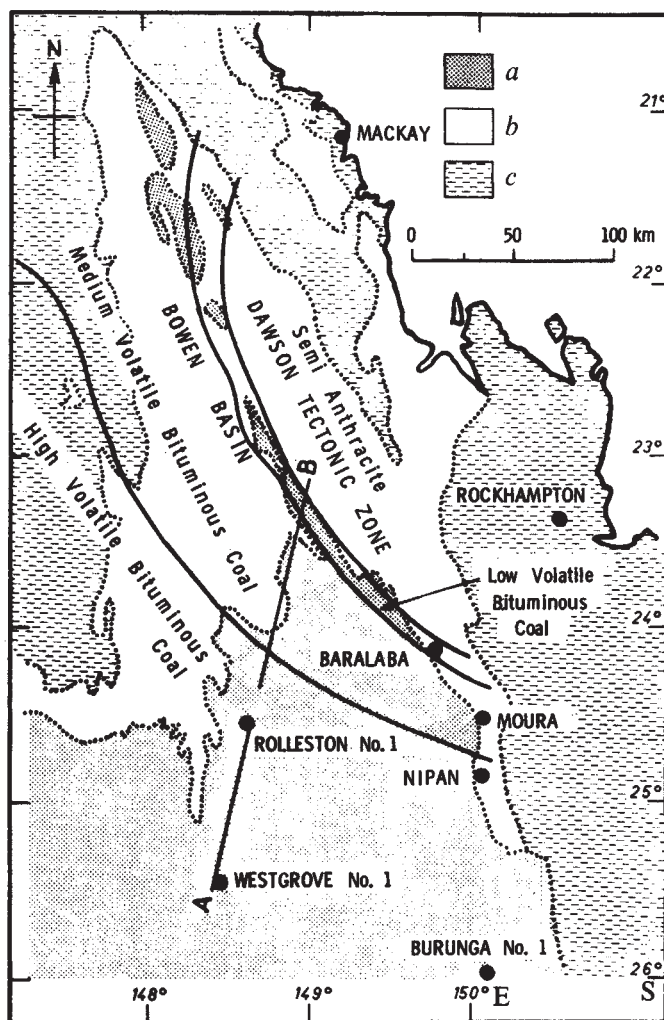


Fig. 1 Coalification trends in the Bowen Basin (after ref. 7). A–B, Location of cross section shown in Fig. 2. a, Mesozoic sediments; b, Permian sediments; c, pre-Permian sediments.

of Triassic sediments. After the deposition of these sediments, the area, particularly the northern part, became uplifted and suffered intense erosion. Contemporaneously, a large synclinal structure formed along the downwarp, the northern part of which is now the Bowen Basin. Tectonic activity was particularly strong along the eastern flank of the Bowen Basin, and the so-called Dawson Tectonic Zone formed. In areas close to this zone, semi-anthracite coals are now exposed at the surface. With increasing distance from this zone towards the south-west, the rank of outcropping coal seems to decrease regularly, down to that of high volatile bituminous coals over a distance of 100 km. This coalification pattern may have been generated by the formation of the Dawson Tectonic Zone (see, for example, ref. 7). Possibly, the tectonic activity together with the deep burial of the coal seams may have been responsible for the increase in rank⁸, though pressure tends to inhibit rather than promote increase in rank⁹. Shibaoka *et al.*¹⁰, however, have shown that temperature increases attributable to deep burial were probably responsible for the increase in rank around the Dawson Tectonic Zone.

No evidence has been given to explain why the degree of tectonic disturbance gradually and regularly decreases away from the Dawson Tectonic Zone—only the zone itself is heavily disturbed. Even if tectonic pressure did decrease regularly over 200 km, it would not affect the coalification over such a distance^{2–6}. The only real effect that the

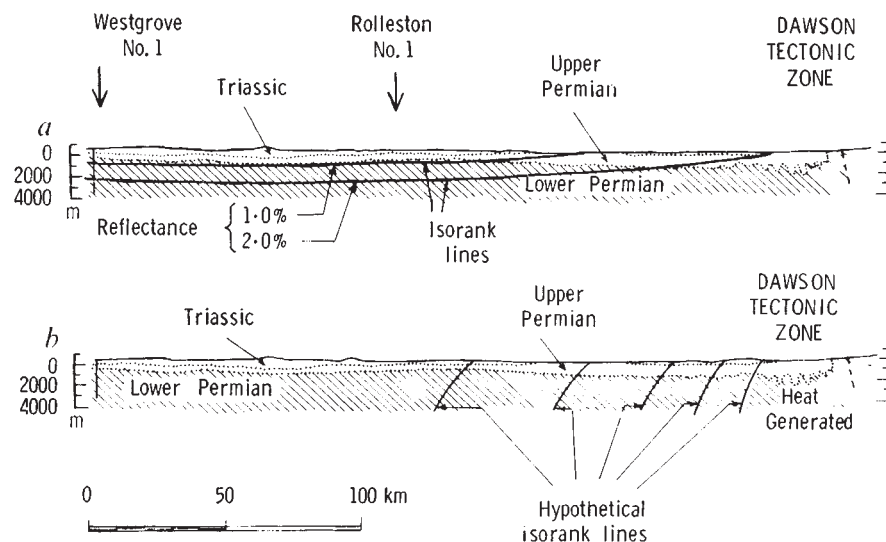
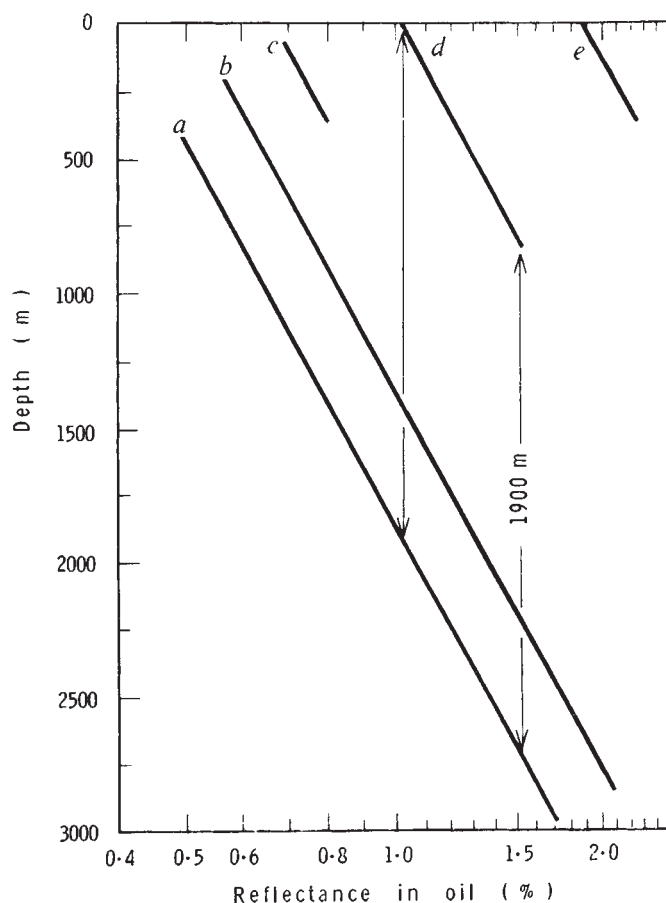


Fig. 2 Reflectance profiles from Westgrove to the Dawson Tectonic Zone showing two alternative models for the isorank lines: *a*, isorank lines controlled by depth of burial followed by uplift and erosion of the western part of the area; *b*, isorank lines drawn assuming a heat source associated with the Dawson fracture.

Dawson Tectonic Zone could have had on coalification must have been attributable to the heat generated during the tectonic activity.

If the zone formed within the space of a few years, which is questionable, the heat generated could possibly have been sufficient to have heated the coal-bearing formations up to at least 200 °C. In that case the isorank planes would incline steeply (Fig. 2*b*) unless the heat source was extremely deep. We have measured the reflectance of coals in boreholes from this area to construct isorank planes. The planes are inclined at an angle of about 1° 30' (3 km in 110 km) between Rolleston and the Dawson Tectonic

Fig. 3 Depth-reflectance curves for coal bores and oil wells in the southern part of the Bowen Basin. *a*, Burunga No. 1; *b*, Rolleston No. 1; *c*, Nipan; *d*, Moura; *e*, Baralaba.



Zone (Fig. 2*a*), thus requiring a local heat source at least 40 km deep. It therefore seems difficult to explain how the geographical variation of coal rank can be attributed to heat generated along the Dawson Tectonic Zone.

A more likely cause of a gradual increase of temperature towards the Dawson Tectonic Zone could have been a thickening of Triassic overburden which has subsequently been lost by erosion. During the Triassic an old land mass lay to the west, and Triassic sediments became progressively thicker towards the depositional centre of the trough which was probably near the present axis of the Bowen Basin.

The Dawson Tectonic Zone may have coincided^{11,12} with the zone of maximum deposition during the Permian and the Triassic. Geological mapping indicates that originally there could very well have been as much as 3 km of Triassic sediments in the central and northern parts of the Bowen Basin. Depth reflectance curves for coal samples from various oil wells suggest that the palaeogeothermal gradient was much the same over the whole southern part of the Bowen Basin, and that where high rank coal is now at a shallow depth, a thick overburden would have been lost by erosion. At Moura, for example, an extra 1,900 m of Triassic sediments could have been lost, compared with a minimal loss at Burunga (Fig. 3). It also seems that a reverse trend may have followed the termination of the downwarp.

We believe that the increase in coal rank in the Bowen Basin was caused neither by tectonic pressure acting on coal seams, nor by the heat generated by the tectonic activity of the Dawson Tectonic Zone. We believe that the main cause was a normal geothermal gradient in thick sediments.

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Desert varnish and marine ferromanganese oxide nodules: congeneric phenomena

BLACK, hydrated ferromanganese oxide (FMO) concretions of microscopic to macroscopic size and complex mineralogy cover ocean floors all over the world at depths of up to 8 km (ref. 1). These amorphous-crystalline nodules have a total Cu, Ni and Co content of 40 p.p.m. to 4% and vary greatly in trace element content, apparently because of varying growth rates caused by the different *Eh* and *pH* in their environments. Black, amorphous, hydrated desert varnish (DV) coatings, 10–100 μm thick, are extensively found in limited areas of the world's arid lands^{2,3} in both the subtropics and in non-sterile areas of the Antarctic dry valleys⁴. Marine FMO nodules are formed principally by biological fixation processes; the mode and rate of formation of DV, on the other hand, are not understood, but concern anthropologists in their efforts to date petroglyphs and other artefacts of ancient man⁵.

The broad similarities in element enrichment shared by FMO forms from terrestrial and marine deserts⁶ (Fig. 1) indicate a shared formation process⁷. Here, similarities between the natural FMO forms are compared to postulate a formation process for DV. This process seems to be started geochemically but later to involve the cation-scavenging properties of colloidal, hydrated MnO_2 (ref. 8) as affected by chemolithotrophic bacteria^{9–11}. All natural FMO forms seem to show broad similarities in elemental composition and nucleation, which should logically lead to a similar mineralogy on subsequent crystallisation, if this occurs.

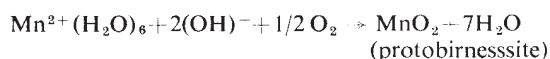
Figure 2 shows the 'genetic classification' diagram of Bonatti, Kraemer and Rydell¹² for lake and marine nodules. Hydrogenous deposits (the group *a*, *b* and *c*) seem to grow extremely slowly, by the precipitation of Fe and Mn from normal seawater in oxidising conditions. In contrast, hydrothermal deposits associated with geothermal activity (group *d*, *e* and *f*) grow quickly, whereas the diagenetic group (*g*, *h* and *i*) forms at varying rates through the remobilisation of Mn from sediments in reducing conditions. Halmyrolytic deposits (not shown) result from the submarine weathering of basalt.

In general, the slowly accreted nodules have high Th/U ratios, transition and trace element levels and degrees of

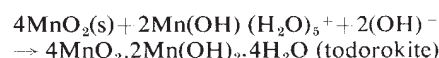
crystallinity. Nodular Ce/La ratios and rare earth element (REE) contents are also inversely proportional to accretion rates¹³, whereas low Mn/Fe ratios and REE depletion are connected with rapid growth in shallow continental margins. The low Co–Ni–Cu content of varnishes places them in the rapidly formed hydrothermal group, *d*, *e* and *f* in Fig. 2. Although Ce has not yet been reported in varnishes, their La/Yb ratio approximates that of Glasby's¹³ 'fast accreted' nodules from Aden and Loch Fyne. Desert varnish has formed in North African and Californian deserts within a period of 25–50 yr (refs 2 and 14).

Burns and Brown¹⁵ have shown by microprobe transection that Fe deposition precedes that of Mn in the marine environment, thus ' MnO_2 ' on coral shows an increasing relative concentration of Mn towards surface. This is also the case for DV¹⁶, with Mn concentration increasing outwards from the leached rock surface as the Fe, Ca, Mg, Ti, Si, Al and K levels decrease. The initial deposition of Fe^{3+} has been attributed¹⁵ to precipitation of $\text{FeOOH} \cdot n\text{H}_2\text{O}$ at local high *pH/Eh* nucleating sites, where weathering has left concentrations of phosphate, silicate or carbonate; precipitation of negatively charged colloidal Mn^{2+} hydroxide, aided by the opposing zero charge potentials of the two hydroxides follows.

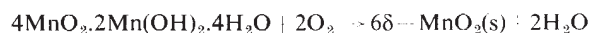
It has been suggested^{15,17} that following nucleation, Mn^{2+} in a high *pH/Eh* marine environment reacts as follows: first, initial deposition



Second, adsorption of more Mn^{2+}



Third, oxidation of todorokite to form $\delta - \text{MnO}_2$

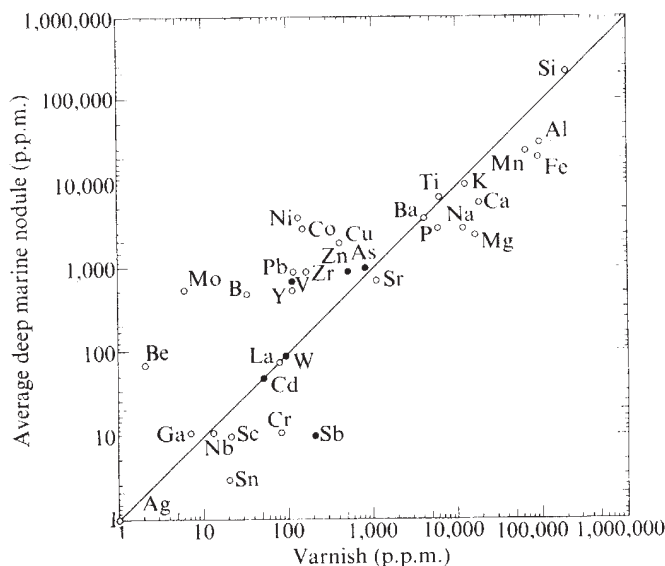


For deep nodules this process eventually leads to the single authigenic high pressure-stable crystalline phase, todorokite, which readily oxidises and dehydrates to yield minute platelets of $\delta - \text{MnO}_2$ admixed with a separate exsolved $\text{FeOOH} \cdot n\text{H}_2\text{O}$ phase. Detrital particles with (—) zero electric potentials, such as quartz, are excluded from the growing nodular surface and are also absent from DV.

Subsequent crystallisation occurs slowly, if at all, with the epitaxial intergrowth of species such as hollandite (Ba , K , Pb , Na)_{1–2} $\text{Mn}_8\text{O}_{16} \cdot n\text{H}_2\text{O}$, psilomelane (Ba , K , Mn , Co)₂ $\text{Mn}_5\text{O}_{10} \cdot n\text{H}_2\text{O}$, birnessite (Ca , Na , Mn)₃ $\text{Mn}_7\text{O}_{14} \cdot 3\text{H}_2\text{O}$ and todorokite (Na , Ca , Mn)₃ $\text{Mn}_5\text{O}_7 \cdot n\text{H}_2\text{O}$, intergrown with polymeric ferrihydrite, $\text{FeOOH} \cdot n\text{H}_2\text{O}$.

These associations of elements suggest (Fig. 1) that the apparently amorphous dioctahedral 2:1 sheet silicate 'lattice' of DV probably contains cryptocrystalline microdomain manganese mineral phases as do the nodules, particularly as Ba appears in all reported DV. 'Goethite' has been reported¹⁴ in one X-ray study of DV, and this work should be extended to obtain a comparison with the known line spectra of the marine nodular phases. These mineral and trace element suites have also been found in Australian desert soil microconcretions¹⁵. Rectilinear semi-log plots of DV Mn against Ba, Co, B, Sn, Y and Yb³ have been obtained in partial agreement with ref. 12, where Mn correlation to Ba and Co in marine nodules is shown. The DV data of Lakin *et al.*³ though scattered, show a similar Mn–Ba–Co correlation. Colloidal scavenging of trace cations is probably involved in the formation both of DV and marine nodules. Murray¹⁹ has shown that precipitated hydrated MnO_2 adsorbs $\text{Co} > \text{Mn} > \text{Ni} > \text{Ba} > \text{Sr} > \text{Ca} > \text{Mg}$, with Co irreversibly sorbed at an alkaline *pH*, whereas manganite sorbs Ni, Ca, Zn, Mg and Ti^{4+} by lattice replacement.

Fig. 1 Average elemental abundance in deep-sea FMO nodules⁶ relative to average desert varnishes (cited). ○, Taken from ref. 2. Averages for Stoddard rhyolite fan, Halloran andesite colluvial, and Sheephead andesite colluvial. ●, From ref. 3. Averages of DV3, 5 and 6.



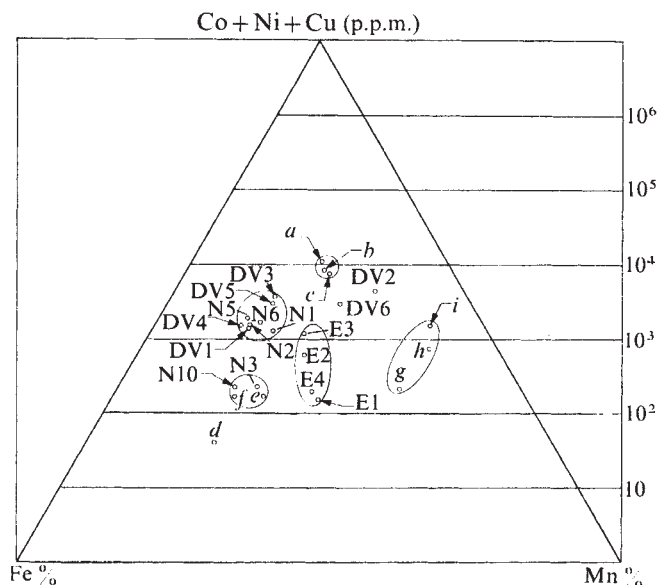


Fig. 2 Ratio Fe:Mn:(Co+Ni+Cu) of representative desert varnishes and hydrothermal and diagenetic marine and lake FMO deposits. Groups *a*, *b* and *c* = hydrogenous; *d*, *e* and *f* = hydrothermal; *g*, *h* and *i* = diagenetic (data of ref. 12). Desert varnishes: DV1–6 and N1, 2, 3, 5 and 6 from ref. 3; E1, Stoddard rhyolite; E2, Halloran andesite; E3, sheephead andesite; E4, Coxcomb tuff of Engel and Sharp².

As noted, nucleation in DV is similar to that in marine nodules but occurs in the alkaline, weathered rock surface where there are silicate, phosphate or carbonate salts. Hem²⁰ has shown that feldspar increases the rate of oxidation of Mn^{2+} to Mn^{4+} , and such phenocrysts may initially be more varnished than the matrix. They may, however, weather more rapidly than the matrix and lose their DV, as is the case for limestone. Dewfall (a major water source²¹ and weathering agent in arid lands) is the likeliest source of the proper amount of moisture to form DV, which is soluble in rain^{5,14}. Other 'constants' in the desert environment are a saline dustfall and an influx of chemolithotrophic microorganisms²². Airborne Egyptian desert dust (~20 μm diameter) contained 18% water solubles, chiefly gypsum and salt²³ whereas airborne Colorado Desert dust was also high in salt, gypsum, lead and iron, with small amounts of Zn and Mn. These conditions are necessary and sufficient for the growth of the ubiquitous desert algal microflora.

Following nucleation in the pre-dawn dewfall, biological growth then takes place during the warming and evaporating cycle. This activity is greatest just below the desert surface and would account for the thick 'groundline'² of varnish found on cobbles in pavement. The groundline band extends from 1 cm above to 3 cm below the soil surface. An exaggerated 'equatorial band' also develops on certain marine nodules at the sediment-water interface²⁴, and this is probably a direct homologue of the DV structure. If desert pavement cobbles are turned over, their unvarnished red undersides become varnished in time, a phenomenon which strongly suggests the phototoxic migration of thermophilic motile, inducible¹⁰ Mn reducers and oxidisers (bacteria and fungi) along the rock surface, probably endosymbiotic with the ubiquitous cyanophytes of desert soils. Stones not in contact with the ground are most heavily DV patinated on upper surfaces and in depressions, which would provide favourable nutrient biological niches based on dustfall. This growth mechanism, and the variables involved, is an extension of that proposed by Scheffer *et al.*¹⁴. As rocks become varnished their blackbody radiation efficiency will also increase thus leading to a thicker DV coating on smaller rocks than on larger ones which cool more slowly. White inert stones such as bull quartz in pavements lack varnish, relative to their neighbours, because they have neither good blackbody radiation

characteristics nor weathering rugosities which trap nutrient dust. Varnishes should be examined for REE anomalies, such as Ce/La, which may apply to their growth rate and age; and they should be examined by means of scanning electron techniques for the surface presence of the admittedly hypothetical bacterial Mn oxidisers.

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Viscosity in the phase transition region of triblock copolymer systems

THE behaviour of melts of styrene-butadiene-styrene (SBS) triblock copolymer under oscillatory shear exhibits a marked departure from that of homopolymers in the low frequency range; the dynamic viscosity $\eta'(\omega)$ increases continually with decreasing frequency, rather than exhibiting the Newtonian limiting value η_0 expected of true fluids¹. This peculiar behaviour has been explained in terms of phase separation which forms a lattice of linked polystyrene domains. Here we present rheological evidence directly demonstrating a thermodynamic transition.

We have studied η' at different temperatures for an SBS block copolymer (Shell TR-41-1467; molecular weight 9,600–47,500–9,400) plasticised to different degrees with dipentene $T_{mpt}=178^\circ C$), a good solvent for both block types. All experiments were carried out on a Weissenberg Rheogoniometer Model R-17, using a platen diameter of 2.5 cm and a 4° cone angle. Readout of amplitude ratio and phase angle was taken from a strip chart recorder at low ω and from Lissajou figures photographed from an oscilloscope at high ω . Temperature control was achieved by means of an electric oven enclosing the platens. Small containers of solvent were placed in the oven to prevent solvent loss by the sample. Oscillation amplitudes were chosen to satisfy the conditions of linear viscoelasticity.

We observed that moderately plasticised systems exhibit a limiting viscosity, η_0 , at low frequencies if the temperature is raised sufficiently but not so high to cause degrada-

tion of the material (a common problem when working with unplasticised polymer). Results for a system with approximately 30% dipentene are shown in Fig. 1. At 25 and 51 °C the low- ω anomaly is seen clearly, as reported earlier for the bulk polymer¹ at higher temperatures, but at 76 °C a Newtonian viscosity is recovered. This transition has never been reported for melts, presumably because degradation problems discouraged work at sufficiently high temperatures.

This change in viscosity behaviour can be explained in terms of the concept of 'separation temperature' (T_s), as proposed by Leary and Williams². At temperatures below T_s the domain system is thermodynamically the more stable state, but above T_s the more stable state is a randomly mixed system behaving as an ordinary liquid with measurable η . The results shown in Fig. 1 would indicate that for

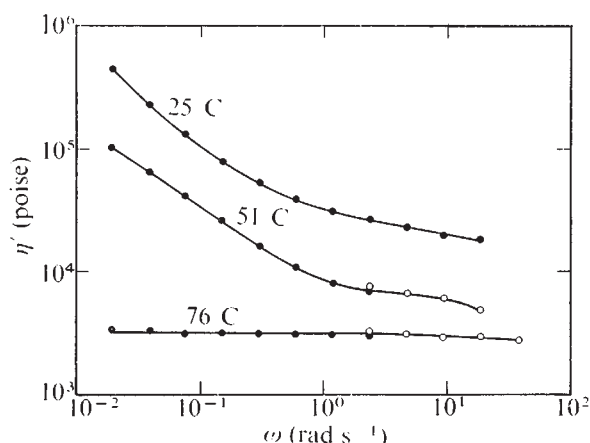


Fig. 1 Dynamic viscosity η' for a styrene-butadiene-styrene block copolymer, with 30% dipentene at temperatures above and below the separation temperature, T_s . ●, From a chart recorder; ○, from an oscilloscope.

this system, T_s is between 51 and 76 °C. A light transmittance experiment with the same system using equipment previously described³ showed a transition in the range 63 to 77 °C, in very good agreement with rheological measurements. Depression of T_s by solvent, as we observed, seems to explain why 'concentrated' solutions (say, 30% polymer) may exhibit no anomalous behaviour at 35 °C (ref. 3).

The rheological evidence tends to support the interpretation and existence of T_s , a concept not universally accepted. Rheogoniometry seems to measure T_s as accurately as previous methods, and identification of T_s values is essential for the design of fabrication processes. Meanwhile, extension of the Leary-Williams model to plasticised block copolymers is in progress⁴.

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Drag reduction in laminar flow

WHEN certain long-chain polymers are added to water, the drag exerted by the solution on fixed boundaries is reduced¹. Although this applies to both internal and external flows, evidence indicates that the effect occurs only in the turbulent regime, at least for steady flow. The work reported here deals with the forced oscillation of a liquid column contained within a semicircular manometer tube, and shows that the addition of a polymer produces a reduction in drag when the flow is laminar.

The apparatus consisted of PVC tubing (3/16 inch bore) arranged in a semicircle (12 inch radius) on a vertical wooden board. The forcing pressure, which is applied to one side of the manometer, is provided by a servo controlled motor which operates rubber bellows through a cam mechanism. The speed of the motor, and thus the forcing frequency, was measured by timing a known number of revolutions of the cam.

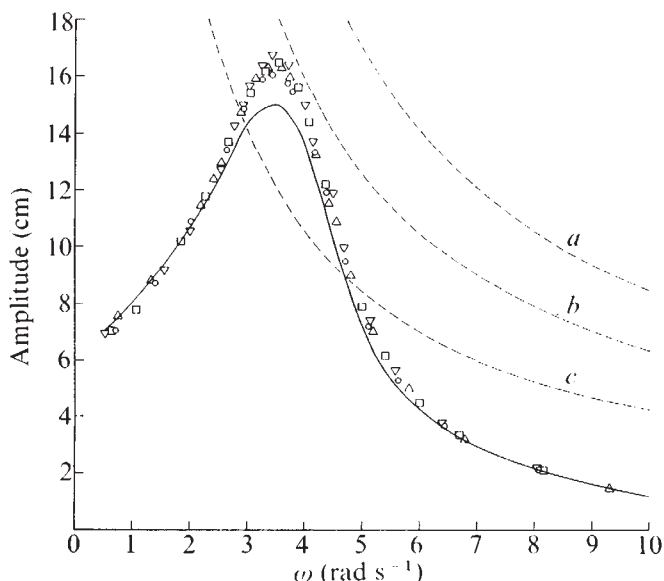
The results are in the form of a resonance test, where the frequency of the forcing pressure is varied and the amplitude of the oscillation of the manometer liquid is measured. Several tests were performed so that the effect of changing the mixture strength could be observed. For each test a new length of tubing was used and the amount of solution in the tube was adjusted so as to preserve a common length of liquid column. Figure 7 shows the results for water and various solutions of Polyox WSR 301 dissolved in water. For the range of solutions tested the polymer produces various degrees of drag reduction, with the weakest solution (12.5 p.p.m.) producing the most effect.

If it is assumed that the displacement of the liquid column is harmonic, then the velocity, and thus the instantaneous Reynolds number, is also harmonic; however, a peak Reynolds number, $\hat{Re}$, can be calculated for any given amplitude and frequency, based on the maximum velocity of the liquid column in any one cycle. Thus, if x is the half-amplitude at a frequency ω , then

$$\hat{Re} = 2\omega x r / \nu$$

where r is the internal radius of the tube and ν the kinematic viscosity of the liquid. Thus, lines of constant $\hat{Re}$ appear as hyperbola in the x - ω plane, as shown in Fig. 1. The results are well within the critical $\hat{Re}$ boundary ($\sim 2,000$) associated with steady flow.

Fig. 1 Amplitude of oscillation as a function of forcing frequency, ω , and mixture concentration. —, water; ○, 100 p.p.m.; □, 50 p.p.m.; △, 25 p.p.m.; ▽, 12.5 p.p.m.; a, $\hat{Re} = 2,000$; b, $\hat{Re} = 1,500$; c, $\hat{Re} = 1,000$.



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The critical Reynolds number for oscillatory flow is frequency dependent², and the parameter which determines the variation from the steady flow case is the Valensi number, N_V , where $N_V = \omega r^2/\nu$.

According to Park and Baird³ the critical Reynolds number increases from 2,000 when $N_V > 30$. In the apparatus described this happens when $\omega > 7$ so that, for the frequency range in which the drag reduction occurs, the critical Reynolds number should still be about 2,000. It would seem that anomalous results are obtained when comparing the reduction of pipe wall drag for steady and unsteady laminar flows.

We draw attention to this because of its possible application. It is a small part of a more fundamental study under the direction of Dr W. D. McComb⁴, to whom grateful acknowledgement is made.

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Knowledge of magnetism in pre-Columbian Mesoamerica

THAT the pre-Columbian peoples of Mesoamerica were familiar with the property of magnetism has been suggested by numerous researchers, among them Coe and Fuson¹. Indeed, a flattened oblong piece of haematite discovered by Coe during the excavation of the Olmec site of San Lorenzo in southern Veracruz state in 1973, has been thoroughly examined by Carlson², who suggests that it probably was manufactured for use as a compass. Fuson has argued that the varied alignments of architectural complexes in many Mayan ceremonial centres may be explained by their having been oriented to compass directions which changed through time, and further, that the Mayas' knowledge of mercury would have permitted them to use vessels filled with this liquid, as well as the water-filled calabashes, to float their 'needles' or lodestones. In January 1975 during field studies at Izapa, in the Pacific coastal plain of south-eastern

Fig. 1 Carvings of snake and turtle heads at Izapa, located 30 m SE of the main pyramid.



Chiapas state, I discovered strong circumstantial evidence that the people of this Late Formative ceremonial centre not only knew about magnetism but possibly even associated it with the homing instinct of the sea-turtle.

While examining astronomical alignments of various of the structures at Izapa, I took a bearing along the axis established by two large sculptures located ~30 m south east of the main pyramid (Fig. 1). In common with the river cobbles that make up the facing of the pyramids and platforms of the site, the sculptures consist of dark brown basalt. Apart from the fact that they are larger and have been specially carved, they are in no way distinguished from the other exposed rock at the site. The smaller of the two sculptures measures 89 cm in length and is unmistakably a representation of a snake head. Some 114 cm behind the snake head, that is, further SE, stands an upright stone stela, roughly squared off but having no discernible

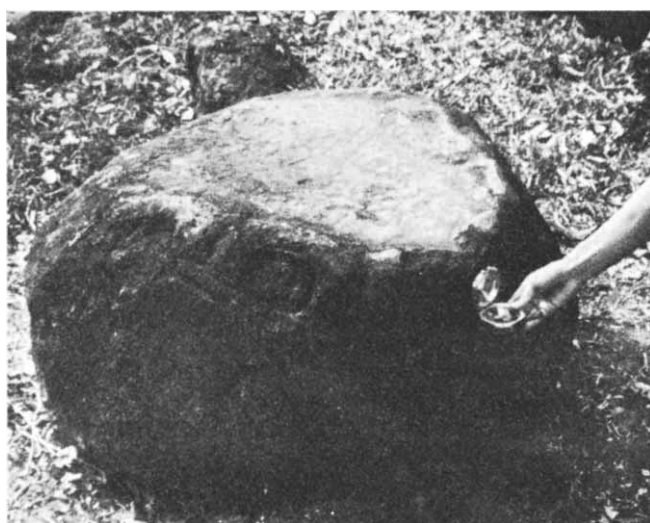


Fig. 2 Close-up of turtle head, with compass needle pointing toward snout.

carvings on any of its faces. A further 256 cm SE is a second stone sculpture, this one measuring 114 cm in length and 122 cm across at its widest point. This stone unquestionably depicts the head of a turtle. When a Brunton compass was brought near the turtle head a sharp deflection of the needle was observed, of more than 60°. No matter where the compass was moved along the perimeter of the sculpture, the needle continuously pointed to the snout of the turtle (Fig. 2). Discovery of this magnetic field prompted the testing of all other exposed rock at the site for magnetic properties, but no others were detected. This would suggest that the Izapans knew about magnetism in that they had reserved a basaltic boulder rich in iron for their carving of the turtle-head, and had executed it so carefully that the magnetic lines of force all came to a focus in the snout of the turtle.

The magnetic turtle-head is not the only representation of this creature found at Izapa. Overlooking the western end of the ceremonial ball court is a large altar carved from a single piece of basalt which is also unmistakably a turtle. A few metres to the south of this altar, adjacent to the wall of the main pyramid, is another sculpture, which has the appearance of an upturned turtle shell, again carved from a single basalt boulder. The latter would obviously have become filled with water during the rainy season, and may well have provided the frictionless surface needed for a shaving or needle of lodestone, floating on a small piece of wood, a leaf, or a straw, to serve as a compass. Clearly the Izapans, a sea-faring people, were impressed by

the navigational ability of turtle³, which are common in this area. It may be interesting to note that the theory that turtles navigate by magnetism has not yet been discounted³.

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Fish kill at low pH in a Norwegian river

THE decline in freshwater fish populations in parts of southern Norway is associated with increasing acidity in rivers and lakes¹. The salmon has been eliminated from many rivers, and hundreds of lakes have lost their trout populations. The chief cause of increased acidity is acid precipitation which is the product of the emission, oxidation and long-distance transport of air pollutants, particularly sulphur dioxide^{2,3}. Similar observations of acid rain and the disappearance of freshwater fish populations have been made in the United States, Canada and Sweden⁴⁻⁶.

Few cases of massive fish kill due to low pH have been documented in natural environments. A massive fish kill in the Tovdal River in southern Norway in the spring 1975 provided an opportunity to investigate physiological changes in the affected population. Formerly a major salmon river, the Tovdal River is now devoid of salmon, *Salmo salar* L., but still has a population of brown trout, *Salmo trutta* L.

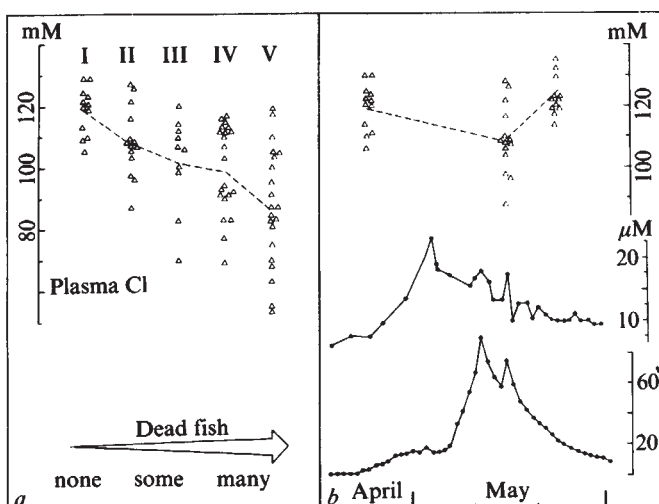


Fig. 1 *a*, Chloride content in the blood plasma from *Salmo trutta* collected from different localities along the Tovdal river. The field stations are ranged in order of increasing fish mortality: station V had many dead fish, station I had none. The fish are a fairly representative sample of the population at each locality. Their weight varied between 17 and 250 g. Blood samples were taken by heart puncture in heparinised syringes within 30 min after capture, and the plasma was separated immediately by centrifugation. Chloride was determined coulometrically on 20- μl samples. The analyses were performed in a mobile field laboratory. *b*, Water flow in the upper part of the river (station I) before, during and after the major snow melting, April 16 to May 30 ($\text{m}^3 \text{s}^{-1}$, lower curve). Acidity of the water was measured as pH and recalculated as μM H^+ (centre curve). Plasma chloride (Δ) from the trout population at station I taken on April 23, May 15 (0 °C) and May 23 (6 °C).

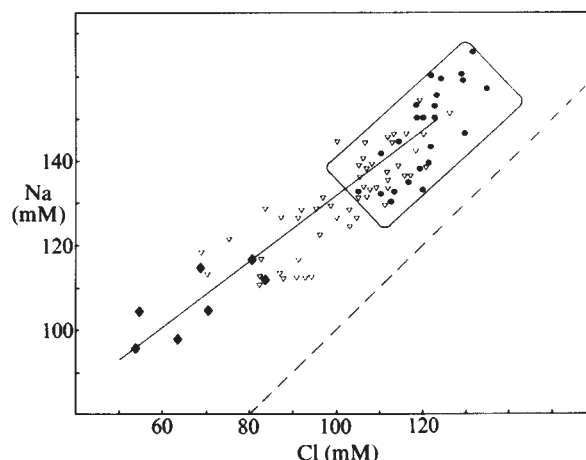


Fig. 2 Plasma sodium plotted against chloride from field localities with different degrees of fish mortality. The frame at the upper right corner indicates animals from station I, with no dead fish. Aberrant behaviour includes: lack of avoidance reaction, spiral swimming, and loss of equilibrium at the moment of capture. Plasma cations were determined by atomic absorption spectroscopy at 1/200 sample dilution. The linear regression line drawn has been calculated: $\text{Na} = 0.746\text{Cl} + 55.35$, $r_{xy} = 0.89$. — — —, Equimolar concentration for sodium and chloride. Temperature 0–6 °C. ●, No dead fish; Δ , many or some dead; diamond, aberrant behaviour.

Salmo trutta L. The catchment area of the river is characterised by highly resistant bedrock, mainly granites and gneisses with thin and poorly developed soils. The valley is sparsely populated and has no industry. When the fish kill was noticed, the river was partly covered with ice and the bottom was littered with thousands of dead trout over a stretch of at least 30 km. The fish kill was apparently associated with an influx of pollutants during early phases of snow melting⁷.

Live trout were captured in shallow water by electro-fishing, and blood samples were drawn immediately. Blood plasma was analysed for Na, K, Mg, Ca and Cl. The fish population in the upper reaches of the river was found to be unaffected, and we were thus able to compare this population with samples of surviving trout from the area of severe fish kill. Figure 1 shows that most of the surviving population at the location with the highest incidence of dead fish (station V) had lower plasma chloride contents than those from the unstressed locality (station I). Fish from localities with fewer dead fish show intermediate values (station II, III, and IV). In Fig. 2 the plasma chloride has been plotted against sodium for all the field data. As might be expected, the decrease in plasma chloride is accompanied by a reduction in sodium. The calculated regression shows that for each chloride ion lost there is a decrease in sodium of 0.75 ions.

At the upper reaches of the river (station I) the snow started to melt on April 21, and continued at a moderate rate until May 6 (Fig. 1b). The increased acidity shown in Fig. 1b during the early phase of melting was accompanied by a similar increase in sulphate and other components associated with acidic precipitation. During this early phase of melting, pollutants accumulated in the snow pack are leached out⁷ and may reach the river unchanged because of frost in the ground. Blood samples taken on May 15, contained significantly less plasma sodium and chloride than samples taken before and after the snow melting. The pH had dropped from 5.2 to a minimum of 4.65 (23 μM) in the early phase of melting, but values as low as pH 4.0 (100 μM) was registered in some of the small tributaries to the main river.

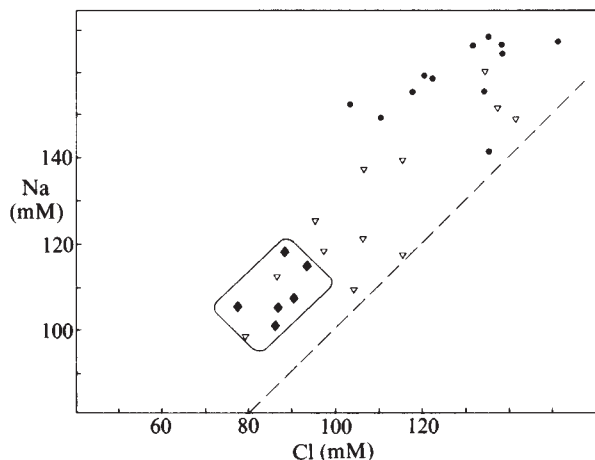


Fig. 3 Plasma sodium plotted against chloride from tank experiments conducted in July 1974 at 18 °C. Trout kept at pH 6.0 and trout exposed to pH 4.0 for 20–100 h. The pH was regulated by metered addition of sulphuric acid. The broken line indicates equimolar concentration for sodium and chloride. "Dying" animals have lost their ability for integrated bodily movements. The frame gives the range in concentration for "dying" animals. ●, pH 6.0; △, pH 4.0; diamond, pH 4.0 "dying".

Fish from a locality with high incidence of dead fish (station V) were transferred to tanks with running water of the same water quality, but neutralised to pH 6.0 by metered addition of dilute potassium hydroxide. After 2 weeks at this pH, the previously stressed fish had regained their normal sodium chloride levels. The change in plasma ions induced by varying only the pH indicates that high acidity is the dominating stress agent for fish in the river.

Brown trout from the wild stock population of the Tovdal River has been used in tank experiments at different times of the year. Fish kept in tanks with water acidified to pH 4.0 lose their ability to regulate plasma sodium and chloride concentrations (Fig. 3). The lowest sodium chloride levels were always found in animals so heavily stressed that the ability for integrated bodily movements had been lost.

Freshwater fish take up ions actively through the gill epithelium. Sodium is exchanged for hydrogen ions and chloride for bicarbonate⁸. Increased hydrogen ion activity in the surrounding medium will impede the active uptake of sodium^{9,10}.

This study has shown that low plasma sodium chloride is associated with massive fish kills and with exposure to low pH in tank experiments and in the field. The plasma content of potassium, calcium and magnesium is not affected. It seems probable therefore that impairment of the active transport mechanism for sodium and/or chloride ions through the gill epithelium is the primary cause of fish death in acid waters. Severe ionic imbalance is known to affect fundamental physiological processes such as nervous conduction and enzymatic reactions. The pH below which fish populations are affected depends on several factors. There are differences between species—salmon is more sensitive than trout. Newly hatched fry are more sensitive than adult fish and lack of recruitment is common in acid lakes^{1,6}.

It should be stressed that the lakes and rivers that have lost their fish populations contain small amounts of dissolved salts. Typical values for specific conductance is 10–30 $\mu\text{mho cm}^{-1}$, total hardness is 0.9–6.0 mg l^{-1} as CaCO_3 and Na is 0.5–2 mg l^{-1} . Not only are such waters poorly buffered but field surveys suggest that the fish population is affected at a higher pH in lakes with extremely low ion content. In the spring, pollutants released in the first phases of snow melt produce a pH shock in the rivers and surface

layers of lakes. The subsequent meltwater lowers the total ion content and thus reinforces the pH stress. The fish kill in the Tovdal River indicates that this is the most critical season for the fish populations.

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Chemical treatment of soil alleviates effects of soil compaction on pea seedling growth

HIGH mechanical resistance of soils, related to naturally occurring hard pans, or to compaction by machinery and untimely cultivation^{1–3}, often reduces crop yields through poor development of root systems^{1,4–6}. In the laboratory, progressive increases in mechanical resistance of the growth medium cause reduced root elongation^{7–9}, stunting and thickening of root systems^{3,10} and reduced shoot growth¹¹. Loosening of subsoil in the field can reduce compaction, increase aeration and waterholding capacity of the soil and improve the distribution of available water¹. Roots can then penetrate to greater depths and crop yields often increase^{1,12,13}. White light also affects root growth, inhibiting axial extension in some species^{14–16}. This effect can be counteracted in cress roots by 3,5-diiodo-4-hydroxybenzoic acid (DIHB)^{17–19}. The fact that compacted soil and white light can both induce stunting and thickening of root systems suggested to us that similar growth control mechanisms might be involved. We have therefore investigated whether DIHB can improve root development in compacted soils; we have shown that this is the case.

The roots and shoots of pea seedlings grown in compacted control soil (moistened with water) in growth room conditions were found to be much shorter and had smaller dry weights than those of seedlings grown in loose control soil (Tables 1–3). Furthermore, the growth-inhibited roots were thickened and bent whereas the roots of seedlings grown in loose control soil were slender and straight. These responses of roots to adverse soil conditions are typical of those observed in various crop plants^{3,8,10,11}.

Addition of 10^{-4} M or 10^{-6} M solutions of DIHB to soil followed by compaction, reduced the level of root growth inhibition, the overall lengths of unstraightened roots being 19% and 31% greater, respectively, than those of corresponding roots in the compacted control soil. When the roots were straightened the chemical was found to have induced increases of 19% and 24% (Table 1). Similar DIHB treatments applied to the loose soil had little detectable effect on root elongation at either 10^{-4} M or 10^{-6} M, root length in both cases being the same as in the loose control soil (Table 2). Clearly, therefore, DIHB enhanced root penetration in compacted soil and was ineffective in loose soil.

Straightening and measuring the roots of pea seedlings grown in 10^{-4} M DIHB compacted soil or the compacted

Table 1 Effect of DIHB solution or distilled water incorporated in a compacted silt loam soil on the length of pea seedling roots

DIHB concentration (M)	Length (mm)	Shoot		Root		% Increase over control	% Increase over control
		Length (mm)	% Increase over control	Unstraightened	Straightened	Unstraightened	Straightened
Control	14.12±0.78	—	—	16.90±0.91	23.97±1.07	—	—
10 ⁻⁶	15.37±1.02	8.85	—	22.12±1.34	29.63±1.36	30.89	23.61
10 ⁻⁴	15.04±0.91	6.51	—	20.06±0.95	28.56±1.12	18.70	19.14

Data are the means and standard errors from six samples each containing at least eight seedlings. Silt loam soil of the Wood Series was taken from the plough layer (0–22.5 cm), sieved (4 mm mesh) and air dried. Gravimetric moisture determinations were carried out so that the final soil moisture content could be adjusted to 20% (pF 3.6) by weight using distilled water or aqueous DIHB solution. Treated or control soil was placed in rigid plastic isodiametric pots (7.5 cm high, 10.2 cm inner diameter, Osma) in four layers of equal weight and each layer was compressed evenly using an air-operated piston. Each pot constructed in this way was filled to a depth of 6.5 cm. Applied pressure at the soil surface was 3.16 kg cm⁻² for compacted soil and 0.15 kg cm⁻² for 'loose' soil (read from a standard curve showing the relationship between soil bulk density and moisture content over a range of applied pressures for the Wood Series soil²⁰), which gave dry bulk densities of 1.5 and 1.1 g cm⁻³ respectively. Seeds of *Pisum sativum* L. cv. Meteor were soaked in flowing tap water for 4 h before germination between sheets of moistened Whatman No. 1 filter paper at 25±1 °C in darkness. After 22–24 h, seedlings with radicles 2 mm long were planted in the pots containing control or DIHB-treated soil. Radicles were embedded firmly in small holes made in the soil surface and the pots were filled to capacity with the appropriate soil, which was gently firmed by the piston at a pressure of 0.15 kg cm⁻². Polythene sheet was used to cover the pots to prevent evaporation of water from the soil. After further incubation for 72 h at 20±2 °C, seedlings were removed from the soil and washed in tap water. Root length of seedlings grown in compacted soil was assessed as (a) the length of a straight line extending from the base of the root to the root tip, thereby not accounting for any deviations due to bending and (b) the entire length of the root after straightening. These two values, which are referred to as unstraightened and straightened lengths respectively, gave a guide to the amount of bending of the root induced by the soil conditions. Roots of seedlings grown in loose soil were free from such bending so only the length of the straight root was recorded.

control soil revealed a 42% increase in length; for roots grown in the 10⁻⁶ M DIHB compacted soil treatment the increase was 34% (Table 1). Since a highly bent root would show a large increase in overall length on straightening and vice versa, it follows that the roots grown in the 10⁻⁶ M DIHB compacted soil were less bent than those from either the 10⁻⁴ M DIHB or the control compacted soils. Thus the concentration of DIHB which was most effective in enhancing root elongation through the compacted soil also reduced root bending in response to high mechanical resistance of the soil.

A trend was detected which indicated that small increases in shoot length occurred when seedlings were grown in compacted soil treated with 10⁻⁴ M and 10⁻⁶ M DIHB, but the chemical had little effect on shoot length in the loose soil treatments (Tables 1 and 2).

Seedling dry weights were not affected by inclusion of DIHB solution in the loose soil treatments, but increases in the weight of both shoots and roots were detected in the DIHB compacted soil treatments (Table 3). The greatest dry weight increase was found in seedlings subjected to the 10⁻⁶ M DIHB compacted soil treatment, which, as we have already seen, was also the most effective treatment for enhancement of root and shoot elongation.

We have demonstrated, therefore, that incorporation of DIHB into compacted soil can produce substantial increases in overall root length of pea seedlings and small increases in shoot length and seedling dry weight. DIHB can thus reduce root growth inhibition induced by both compacted soil conditions and exposure to white light. This supports our initial idea that the two responses might have similar control mechanisms. Investigations in our laboratories have shown that DIHB may counteract effects of light on root

elongation by reducing ethylene-induced growth inhibition^{19,21}. Ethylene can also cause swelling and bending of roots^{22,23} and these distortions are similar to those observed in roots of seedlings growing in compacted soils^{3,10}. Anaerobic conditions arising as a result of conditions prevailing in some compacted soils can stimulate the production of ethylene by soil microflora^{20,24,25} and mechanical resistance can also induce endogenous production of ethylene by roots and shoots^{23,26}. Thus, it is possible that DIHB acts by reducing the production and/or the inhibiting effects of ethylene derived from the soil or the plant tissue or both. Further research is required to assess the relative

Table 3 Dry weights of root and shoot tissues of pea seedlings treated with DIHB

DIHB concentration (M)	Dry weight per seedling (mg)			
	Shoot	Root	Shoot	Root
Control	8.41±0.72	5.54±0.52	9.77±0.45	7.66±0.31
10 ⁻⁶	10.99±1.17	6.50±0.72	9.96±0.35	7.92±0.18
10 ⁻⁴	9.55±0.86	6.03±0.53	10.14±0.15	7.87±0.08

Pea seedlings were grown for 72 h at 20 °C in 'loose' or compacted silt loam soil, treated with DIHB solution or distilled water (control) to give a 20% soil moisture content. Data for each treatment are the means ± s.e. from six samples, each consisting of at least eight seedlings.

importance of mechanical resistance, ethylene and anaerobic conditions in limiting seedling growth, and also to determine whether DIHB relieves growth inhibition due to one or more of these factors. The moisture content of the soil used in the present studies, however, was well below that at which pea seedling growth is limited through lack of aeration^{9,26} and in view of the short growth periods involved it seems unlikely that anaerobic conditions would be established. Whatever the reason, the important aspect of our findings is that seedling growth can be improved by treating compacted soil with low concentrations of specific chemicals, an effect which is not apparent in non-compacted soil.

Intensive investigations are now in progress to determine the conditions necessary for optimum plant response to DIHB and the precise mode of action of the chemical and its analogues. 3,5-Dichloro- and 3,5-dibromo-4-hydroxybenzoic acids, for example, have much the same effect on pea seedling growth in compacted soil as DIHB and the results of these studies will be published in full elsewhere.

Table 2 Effect of DIHB solution or distilled water incorporated in a 'loose' silt loam soil on the elongation of pea roots and shoots

DIHB concentration (M)	Shoot		Root	
	Length (mm)	% Change over control	Length (mm)	% Change over control
Control	21.47±0.56	—	65.98±1.82	—
10 ⁻⁶	21.37±0.46	-0.47	64.55±1.59	-2.17
10 ⁻⁴	21.24±0.49	-1.07	67.04±1.44	+1.60

The density of the soil was 1.1 g cm⁻³, and its moisture level was 20%. Seedlings were grown for 72 h at 20 °C and the data for each treatment are the means ± s.e. from six samples each consisting of at least eight seedlings.

Clearly, these findings of enhanced seedling performance in adverse soil conditions could open exciting new possibilities in practical agriculture by helping to combat the effects of soil compaction on plant growth using chemical rather than mechanical means.

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Substance stimulating the differentiation of spores of the blue-green alga *Cylindrospermum licheniforme*

THE study of intercellular inductions in higher plants and animals is hindered by the complexity of the multicellular tissues involved. Sporulation of filamentous blue-green algae is a relatively simple process to study because these algae contain, in linear array, only three types of cells. There are vegetative cells, and heterocysts and spores which form by differentiation of vegetative cells. Wolk^{1,2} showed that heterocysts of the blue-green alga *Anabaena cylindrica* Lemm. induce adjacent vegetative cells to differentiate into spores. We report here that a substance produced by the related alga, *Cylindrospermum licheniforme*, greatly stimulates sporulation of that same alga.

Stock cultures *C. licheniforme* Kütz. were grown axenically³ in an eightfold dilution of the medium of Allen and Arnon⁴ on a rotatory shaker at $27 \pm 1^\circ\text{C}$, illuminated with cool-white fluorescent light at an intensity of 2,200 lx. These cultures, subcultured 1% v/v weekly to maintain a density below $2.5 \mu\text{g}$ chlorophyll per ml, contained no spores.

Phosphate deficiency stimulates sporulation of *Cylindrospermum*⁵. Cultures of *C. licheniforme* sporulated in phosphate-free standard sporulation medium (SSM)¹ containing 5.7 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid (TES), pH 7.5, as buffer in place of DL-alanyl-glycine. Conditioned SSM was the centrifugal supernatant fluid from 50 ml 10–15-d-old cultures, and from 250 ml

15–20-d-old cultures. SSM and conditioned SSM were inoculated (2%, v/v) from 7-d-old stock cultures, and were cultured in the same conditions of temperature and light as were the stock cultures. In some experiments, conditioned SSM was supplemented with all the constituents of SSM, or was lyophilised and then made up to its original volume by addition of SSM or water.

In *C. licheniforme*, heterocysts normally develop at the ends of filaments, and spores differentiate first next to the heterocysts. The extent of sporulation was quantified as the percentage of heterocysts with an adjacent spore or spores. (Heterocysts can be caused to form at an intercalary position⁷, in which case spores form on both sides of these heterocysts, but intercalary heterocysts were not observed as part of our experiments.) Cells were considered to be heterocysts only if they contained the polar granule⁶ characteristic of heterocysts, and were considered to be spores only if they were at least twice as long as a normal vegetative cell, and showed no sign of septation. Growth was measured as an increase in the chlorophyll content of a culture, as determined with methanolic extracts⁷.

Filaments grown in SSM show no acceleration of sporulation until the fifth day of culture, whereas the acceleration of sporulation of filaments grown in conditioned SSM starts essentially immediately (Fig. 1). The final percentage sporulation is approximately the same in the two cultures. Growth and sporulation of *C. licheniforme* in SSM therefore modifies (conditions) that medium in a way that makes it stimulatory to spore formation on reinoculation. No significant difference in growth accompanies the difference in sporulation. Therefore, stimulation of sporulation cannot be due to an effect on growth. Conditioning also had no significant effect on the pH of SSM.

Supplementation of conditioned SSM with all the constituents of SSM does not affect the sporulation–stimulation which results from conditioning (Table 1). Supplemented, conditioned SSM contains all of the components of SSM in concentrations equal to or greater than their concentration in SSM. The stimulation of sporulation which is effected by conditioning of SSM is not, therefore, the result of a depletion of the medium. Rather, the filaments produce a substance which is released into the medium and which stimulates the differentiation of spores in newly inoculated filaments. We conjecture that sporulation of the original inoculum results from an increasing concentration of this same substance.

A 1:19 dilution of conditioned SSM with SSM retains 50% of its sporulation-accelerating activity. Lyophilised samples retain activity after storage for at least 1 month at room temperature, reconstitution with water or SSM, and sterilisation by autoclaving.

Blue-green algae release a large amount of polypeptidic and other organic material into their medium^{8–12}. The substance produced by *C. licheniforme* may function normally as an extracellular agent, an intrafilamentous agent, or both. As an extracellular agent, it might become more concentrated during stagnation or desiccation of a natural body of water, and thereby signal the need for

Table 1 Effect of supplementing conditioned medium with components of fresh sporulation medium

Medium	% Heterocysts with spores	
	Experiments	
	A	B
Standard sporulation medium (SSM)	6.4 ± 2.7*	5.8 ± 2.6*
Conditioned SSM	66.0 ± 6.1	64.8 ± 6.6
Conditioned, supplemented SSM	68.0 ± 13.8	67.2 ± 12.8

In experiments A, constituents of SSM were added to liquid, conditioned SSM. In experiments B, liquid SSM was added to lyophilised, conditioned SSM.

* Mean ± s.e., from five experiments, each of which was assayed after 3 d of culture.

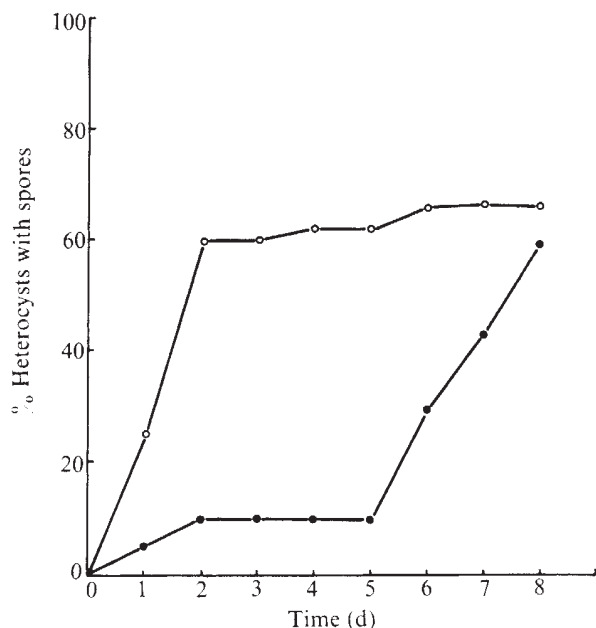


Fig. 1 Time course of sporulation of *C. licheniforme* in SSM (●) and in SSM conditioned by previous growth and sporulation of the alga (○).

C. licheniforme to sporulate so as to survive an impending period of dryness. Within filaments, the substance may be involved in the possible induction, by heterocysts, of the sporulation of adjacent vegetative cells¹³. Thus, if heterocysts induce adjacent vegetative cells to sporulate by producing this substance, and if some of it were to leak out, the exogenous supply could accelerate sporulation in a second inoculum by supplementing the supply, within vegetative cells, which comes from heterocysts. Alternatively, the juxtaposition of the first-formed spores to heterocysts may mean that the heterocysts control the entry of, activation of, or response to, the exogenous factor.

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Inference of the time of origin of some *Drosophila* species

Much attention has recently been paid to the interpretation of protein evolution through amino acid substitution. These events are under genetic control and seem, in certain well documented cases, to occur at a constant rate per site per

year for many species^{1,2}. At the population level, arguments have been adduced that the observed allozymic variation may also have similar clock-like properties³. A further area of present interest is the existence of certain pairs of species which show only very slight genetic difference as measured by allozymes^{4–6}. These cases may represent pairs of newly formed species. Thus, genetic similarity may serve as direct measure of the recency of the cladistic event which separated the two compared lines of descent. This note extends the 'protein clock' idea to a series of species endemic to a set of successively younger island volcanoes, for which ages are well documented.

The ages of each of the twelve volcanoes comprising the five largest Hawaiian Islands (Kauai, Oahu, Molokai, Maui and Hawaii; see inset, upper left, Fig. 1) are accurately known from potassium-argon and magnetic declination data⁷. According to a well-supported theory, each volcano has been formed in sequence from north-west to south-east. They have emerged above the ocean in the order listed above, as the Pacific crustal plate has drifted north-westwards over a fixed 'hot spot' in the Earth's mantle⁸. The ages of the oldest rocks of each volcano⁷ can be read along the horizontal axis in Fig. 1.

Eight very closely related but morphologically distinct (that is, non-sibling) species of the *Drosophila planitibia* subgroup are found on these islands^{9,10}. They show very narrow geographical distributions, being restricted to the volcanoes listed under each species name on Fig. 1. The island of Hawaii shows no lava flows older than 700,000 yr (ref. 11), so the two sympatric species endemic to the island of Hawaii must be relatively newly formed. These species, *D. heteroneura* and *silvestris*, are, moreover, virtually indistinguishable electrophoretically⁵. *D. heteroneura*, probably the newest species in the series, has been selected as a standard for pairwise comparisons with each of the other seven species. Genetic similarities based on allozymes (Rogers¹² coefficient *S*) have been used in these comparisons.

Along the ordinate in Fig. 1 is given the genetic distance, *D*, obtained in pairwise comparisons between *heteroneura* and each of the other seven species. Following Nei's¹³ suggestion, *D* has been calculated as the negative natural logarithm of the similarity coefficient. Differences calculated from the data⁵ have been plotted against time, measured by the age of the oldest known emergent rocks of each volcano or island. The time of each cladistic event (splitting) is indicated along a diagonal line, *A*, in Fig. 1. This line assumes allozymic identity at separation and then an accumulation of genetic difference directly proportional to the elapsed time since the cladistic event. Diagonal *A* assumes that genetic difference accumulates at a rate of 1% in 20,000 yr. These are large, slow-breeding flies of high, cool rain forests; there may be no more than two generations a year.

These assumptions fit the age of the islands, the endemic position of the species and the chromosomal phylogenies reasonably well. For example, the cladistic event separating *D. substenoptera* of Oahu from the line leading from *picticornis* of Kauai towards *heteroneura* would have occurred when Kauai and West Oahu (Waianae) were the only existing emergent volcanoes. *D. neopicta*, a pivotal ancestor common to the more advanced species in the chromosomal phylogeny¹⁰, would have been, as expected, the first of the series to colonise Molokai. At first glance, *hemipeza* seems, from the electrophoretic data alone, not to be old enough to have evolved ancestrally on Oahu. This is, however, in accord with earlier theories based on chromosomes^{9,10}. Thus *hemipeza* has been considered a 'back-migrant', having colonised Oahu relatively recently from a Molokai ancestor.

Diagonal dashed lines *B* and *C* (Fig. 1) assumes a slower and faster rate of change respectively. *B* shows an accumu-

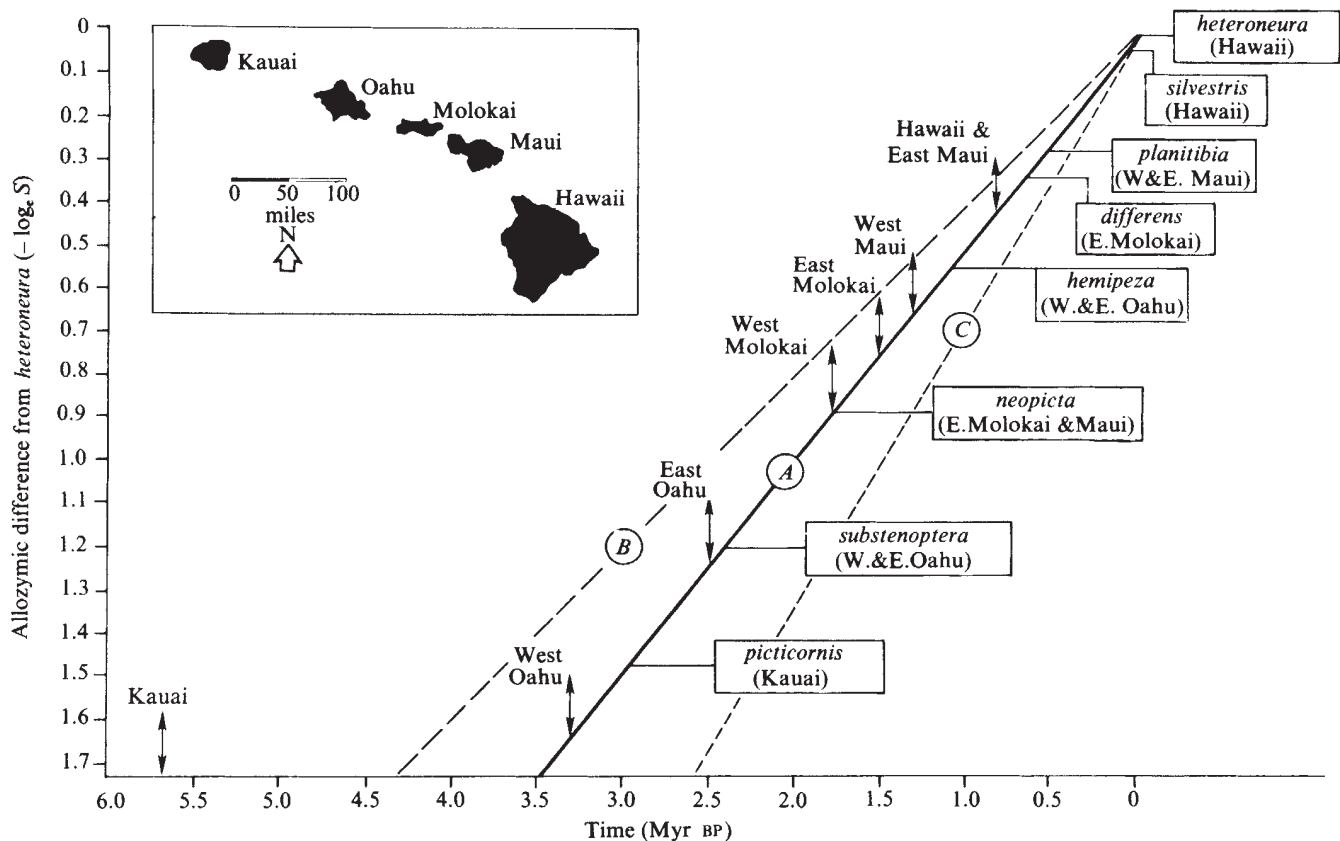


Fig. 1 Allozymic difference and time of species origin in eight species of Hawaiian *Drosophila* (*planitibia* subgroup).

lation genetic difference at the rate of 1% in 25,000 yr and C a rate of 1% in 15,000 yr. Both of these assumptions produce a less satisfactory fit of the data on differences to the geological, geographical and chromosomal data. The rate which fits best, suggests that a level of $D=1.0$ may be attained after two million years of separation. This is about 15 times faster than the rate suggested by another well-documented case⁶.

Accordingly, the following cautious suggestion may be made. Accumulation of electrophoretically detected protein differences over geologic time may possibly serve as a useful clock mechanism, permitting details of the past evolutionary history of a species to be inferred from the biochemical state of the living forms.

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Positional signalling by mouse limb polarising region in the chick wing bud

THE development of the chick limb may be viewed in terms of positional information; that is, the spatial pattern of cellular differentiation results from the cells interpreting their position in a three-dimensional coordinate system¹. We have presented evidence that positional information along the antero-posterior axis is specified by a signal from the zone of polarising activity (ZPA), which is at the posterior margin of the wing bud^{2,3}. Grafts of ZPA to anterior regions of an early bud result in mirror image duplication between grafted ZPA and host ZPA which is particularly evident in the pattern of the digits. The three digits of the chick wing⁴, digits 2, 3, and 4 appear to be specified by their distance from the nearest ZPA, digit 4 being closest, then digit 3 and then digit 2. The ZPA from the leg can also specify additional wing digits when grafted into the wing which suggests that the positional information along the antero-posterior axis in leg and wing is similar, but that interpretation differs. We therefore wanted to know whether a ZPA was present in other classes of vertebrates, and if so whether the signal from it was also similar. We investigated this question by grafting tissue from embryonic mouse limbs into developing chick wing buds.

Arms and legs of mouse embryos aged 10 d (the day of the vaginal plug was taken as day 0) were used to provide tissue to graft into the chick wing. These limbs were chosen as, morphologically⁵, they resemble stages of the chick wing which have been shown to possess a ZPA. As far as we can judge from the work of Jurand⁶, the time taken to lay down the pattern of the forelimb is approximately the same for both chicks and mice. The arms and legs were cut off

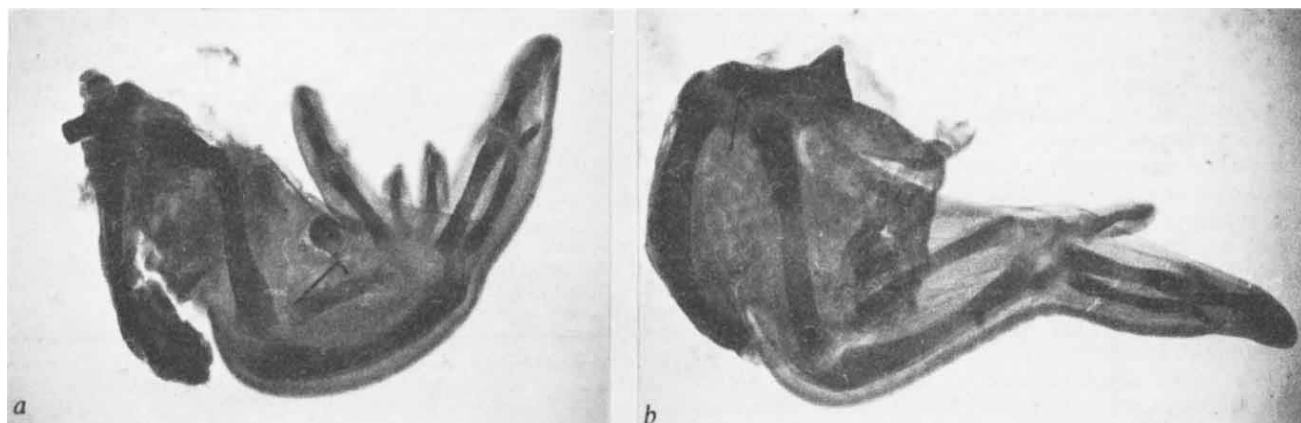


Fig. 1 Dorsal views of whole mounts of operated wings stained to show cartilage pattern. *a*, Wing resulting from a graft of a mouse arm ZPA showing a pattern of digits 32234. Note there is a small nodule of cartilage at the anterior margin of the wing, but not an identifiable digit 4. *b*, Wing resulting from a graft of a piece of tissue from the anterior margin of a mouse arm showing a normal pattern of digits, 234. The little hooks are the pins used to keep the grafts in place.

the mouse embryos, and regions from posterior margins of the limbs $\sim 300\ \mu\text{m}$ from the tip were tested for ZPA activity. This region was taken because it would correspond to the ZPA of the chick limb bud. These small pieces of mouse limb tissue were pinned into holes cut into the anterior margins of embryonic chick wings (stage 20⁺22) opposite somite 16 in contact with the apical ectodermal ridge. (In this position with an implanted chick ZPA the pattern of digits in the resultant wing has been shown³ to be 432234 or 43234.) In a few cases the graft was placed opposite somite 17. The host chick embryos were then incubated for a further 5 d at 37 °C; then they were killed and the pattern of the digits of the operated wing was examined. From 20 grafts of these regions from mouse limbs, we obtained 13 wings with extra digits; 5 wings with normal digits; and 2 wings with extra pieces of cartilage which could not be identified as particular digits. Of the 13 grafts which produced wings with extra digits, 6 wings had the digit pattern 2234 and the remaining 7 wings 32234 or 3234 or 334 (see Fig. 1*a*). Regions of both arms and legs of mouse embryos were used in these experiments, and there was no detectable difference in the ability of these to specify additional digits in the wing. As a control, we took equivalent pieces of tissue from the anterior margin of mouse legs and arms and grafted these into chick wings in the same way. All 7 of these wings were normal, with no extra digits (see Fig. 1*b*). In five additional grafts, more proximal pieces of tissue from the posterior margins of the mouse limbs were assayed and these operated wings were normal.

These results show that the embryonic mouse limb possesses a ZPA and its position is similar to that in the embryonic chick wing. Moreover, this mouse ZPA can specify additional digits in the chick wing. We would emphasise that the response to a grafted ZPA has thus far not been obtained by grafting any other tissue, even though a wide variety have been tested. Such test tissues include: tissues from other regions of the chick limb, other embryonic chick tissues such as liver and brain, and rat endocrine organs.

The pattern of digits specified by the mouse ZPA must be compared with that specified by a chick ZPA. There was no way of predicting the effect of the mouse ZPA in the chick limb, since we know neither the relative level of the signal, nor the threshold for specifying the various digits of the mouse. Whereas with a chick ZPA opposite somite 16 one almost always obtains a well formed digit 4 adjacent to the graft³, with the mouse ZPA this never occurred. There is reason to believe that the signal from the ZPA is graded, and falls off with distance³. Whenever we have effectively reduced the signal, by placing the chick

ZPA further away from competent tissue, or by interfering with its propagation by Nucleopore filters, digit 4 is not formed, but digits 3 and 2 may develop. Thus the inability of the mouse ZPA to specify digit 4 may be caused either by its specifying an intrinsically lower positional value, or by the transmission of the signal being impaired.

Slack (personal communication) has recently shown that there is a region similar to the ZPA in early amphibian embryos, and one may thus reasonably infer that a ZPA is present in all vertebrates with limbs. Our results suggest that the signal from it may be the same in all vertebrates, the differences along the antero-posterior axis being due to differences in interpretation. In this connection it is of interest that mouse dermis can interact with chick epidermis to produce feather buds from the epidermis^{7,8}, and Waddington⁹ showed that Hensen's node from a rabbit gave rise to a secondary neural tube when implanted into a chick host of the same developmental stage.

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Suppression of the incidence of death with spontaneous tumours in DBA/2 mice after *Corynebacterium parvum*-mediated rejection of syngeneic tumours

KILLED *Corynebacterium parvum* has been effective when administered into tumours or when included as a subcutaneous inoculum containing tumour cells (the subcutaneous site, unlike the intradermal site¹, facilitates early

tumour cell dissemination)²⁻⁴. After tumour rejection, the augmented host resistance prevented progressive growth of potentially lethal metastases present at the time of therapy⁵. In addition, animals rejecting tumours formed from primary grafts of tumour cells admixed with killed *C. parvum* (compared with those rejecting tumours after intratumour therapy with *C. parvum*) exhibited an augmented tumour-specific protection against inoculation of large challenge cell doses⁶. Whitmire and Huebner⁷ reported a significant decrease in incidence of 3-methylcholanthrene-induced malignancies in C57BL/Cum and BALB/c C_v mice after preimmunisation with formalin-killed wild-type virus and Rauscher leukaemia virus vaccines administered with Freund's complete adjuvant. We have now found a considerable decrease in the incidence of spontaneous tumours in the high-incidence strain of DBA/2 mice after rejection of tumours formed from subcutaneous transplants of syngeneic mammary adenocarcinoma cells admixed with killed *C. parvum*.

DBA/2 female mice (4-6 weeks old, Jackson Laboratory) and the well differentiated syngeneic T1699 mammary adenocarcinoma (Jackson Laboratory) were used. Seven hundred and fifty previously unsensitised mice were separated into five equal groups and received subcutaneous injections as described in Table 1. We included 60 unsensitised mice which received 10⁶ living tumour cells alone. The experiment was repeated 3 months after the start of the initial studies. In addition (Table 2) three groups of 50 unsensitised mice received subcutaneous injections as for groups 1, 4 and 5. They were evaluated for tumour-specific resistance.

Of the five groups (Table 1), mice receiving subcutaneous living tumour cells and killed *C. parvum* (group 5) developed tumours at the injection site. Tumours grew for 11 d (mean) followed by their rejection in all animals as previously described². Only four of the 150 mice in group 5 developed spontaneous tumours (mammary adenocarcinomas) at 10-13 months of age. They exhibited minimal increase in tumour size (from the day of origin) for approximately 60 d (mean) followed by accelerated tumour growth and death with growing tumours and metastases (survival range from onset of tumour was 77-96 d). Mice given irradiated tumour cells mixed with *C. parvum* (group 4) exhibited no tumour growth at first but 49 developed spontaneous tumours at 9-13 months (with growth patterns

Table 1 Incidence of death from spontaneous tumours in DBA/2 mice

Group (150 mice per group)	Deaths from spontaneous tumours	
	Experiment 1	Experiment 2
(1) 0.9% NaCl	105	117
(2) Killed <i>C. parvum</i> (200 µg) alone	96	107
(3) Irradiated T1699 cells (10 ⁶) alone	101	112
(4) Irradiated T1699 mixed with killed <i>C. parvum</i> (200 µg)	49	56
(5) 10 ⁶ Living T1699 cells mixed with killed <i>C. parvum</i> (200 µg)	4	9
10 ⁶ Living T1699* cells (60 mice)	60	60

*All animals died with growing tumours and metastases after injection of tumour cells.

Effect of *C. parvum*-mediated rejection of T1699 mammary adenocarcinoma cells on death with spontaneously growing tumours in DBA/2 mice. Groups of DBA/2 mice (150 animals each) received subcutaneous injections (0.2 ml) of one of the following (1) 0.9% NaCl; (2) killed *C. parvum* (200 µg) and 0.9% NaCl; (3) 10⁶ irradiated T1699 tumour cells (10,000 r.); (4) 10⁶ irradiated T1699 cells with killed *C. parvum* (200 µg), and (5) 10⁶ living T1699 cells with killed *C. parvum* (200 µg). A further 60 mice received 10⁶ living T1699 cells alone. The animals were inspected weekly for 30 months or until death with or without growing tumours. All animals receiving living tumour cells mixed with killed *C. parvum* exhibited tumour growth (at injection site) for 11 d, followed by tumour rejection. Mice receiving tumour cells alone died with growing tumours and metastases.

Table 2 Tumour-specific resistance in DBA/2 mice

Initial injection	Second injection (5 mice per group) Live 10 ⁷ tumour cells	Survival after second sub- cutaneous injection (%)
(1) NaCl (0.9%)	T1699 cells CAD ₂ cells	0 0
(4) 10 ⁶ Irradiated T1699 tumour cells mixed with killed <i>C. parvum</i> (200 µg)	T1699 cells CAD ₂ cells	100 100
(5) 10 ⁶ Live T1699 tumour cells mixed with killed <i>C. parvum</i>	T1699 cells CAD ₂ cells	100 0

Tumour-specific protection in DBA/2 mice after *C. parvum*-mediated rejection of T1699 tumours. At 20 months, 10 surviving animals from each of the above three groups were separated into two subgroups of five mice each. They then received subcutaneous injections (0.2 ml) of either 10⁷ T1699 or 10⁷ CAD₂ cells.

similar to those in group 5) followed by death with growing tumours and metastases (range of survival after onset of tumour was 70-102 d). The remaining three groups had higher incidences of spontaneous tumours. Of these, 105 treated with 0.9% NaCl (group 1) developed tumours at 8-15 months and died with growing tumours and metastases (range of survival from onset of tumour was 40-60 d). The groups treated with killed *C. parvum* (group 2) or irradiated tumour cells (group 3) exhibited similar incidences of spontaneous tumours and survival after onset of tumour (range: *C. parvum*, 32-35 d; irradiated T1699 cells, 44-67 d). Findings were similar in experiment 2. Histological sections from random autopsies revealed that all tumours were mammary adenocarcinomas with various degrees of differentiation. The 60 mice receiving 10⁶ living T1699 cells alone (in experiments 1 and 2) all died with growing tumours and metastases between 28 and 48 d after inoculation of tumour cells.

At 20 months of age, 10 surviving animals (Table 2) from each of the three groups (50 per group) given treatments approximate to groups 1, 4 and 5 were separated into two subgroups of five. All mice of a subgroup received subcutaneous injections of either 10⁷ live T1699 or 10⁷ CAD₂ cells. The CAD₂ mammary adenocarcinoma syngeneic to DBA/2 mice is antigenically different from the T1699 tumour. The characteristics of this tumour which originated spontaneously in a DBA/2 female have been described before⁸. The results (Table 2) show that the animals that did not develop spontaneous tumours (after rejection of tumours formed from grafts of T1699 cells admixed with *C. parvum*: group 5) were protected and did not develop tumours after reinjection of 10⁷ live T1699 cells. Findings were similar when the surviving animals from group 4 received a second injection of T1699 cells. But, the subgroups of mice in groups 4 and 5 (Table 2) that received a second injection of 10⁷ living CAD₂ cells were not protected and died with growing tumours and metastases as did the mice in group 1 which received either CAD₂ or T1699 cells. All surviving mice (experiments 1 and 2) were dead at the end of 30 months with no tumours.

Thus DBA/2 mice exhibited a 3-6% incidence of death from spontaneous tumours after rejection of tumours formed from grafts of syngeneic mammary adenocarcinoma cells mixed with killed *C. parvum*. Furthermore, mice given irradiated syngeneic tumour cells mixed with killed *C. parvum* exhibited a 50% reduction in that incidence of death with spontaneous tumours compared with the control groups. In addition, mice given either irradiated or living tumour cells mixed with killed *C. parvum* exhibited tumour-specific protection which was evident for 20 months suggesting that the conferred host resistance was of long duration.

These findings were probably associated with the immunostimulant adjuvant properties of *C. parvum*

administered in association with living tumour cells. Thereafter, the augmented response conferred as a result of rejection of a large mass of tumour may have been directed by both virus-specific and tumour-specific transplantation antigens associated with these tumour cells and continued to protect the host from death with spontaneously growing tumours, or perhaps prevented progressive growth of newly formed tumour cells *in vitro*. Evidence for this comes from the demonstration that pretreatment of experimental animals with these viral or tumour antigens protected the recipients against tumour growth from syngeneic cell grafts⁹⁻¹³. Considerably less protection was afforded the mice treated with irradiated tumour cells mixed with killed *C. parvum*, which may have reflected a weakened response towards a decreased tumour cell antigen mass, but may correlate with studies showing that optimal usefulness was observed in experimental animals when living tumour cells were used in specific active immunotherapy^{17,18}.

The immunopotentiating properties of *C. parvum* in association with tumour cells also conferred augmented host resistance¹⁹. This vaccine when administered to experimental animals activated both the reticuloendothelial system and phagocytosis for long periods. In addition stem cells were stimulated to differentiate into macrophages in these animals; T and B cells were stimulated to proliferate and recruitment of lymphocytes was stimulated²⁰⁻²². *C. parvum* also stimulated the primitive surveillance mechanisms that have been found to be effective in tumour rejection^{19,23,24}.

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Immunosuppression by micromolecular fibrinogen degradation products in cancer

PATIENTS with advanced cancer and lymphoid malignancies often show impaired cellular and humoral immune responses, partly caused by serum factors inhibiting lymphocyte reactivity¹⁻³, which, although not fully defined, are considered to be low molecular weight (poly)peptides.

Another frequent finding in malignant diseases is the appearance of circulating fibrin(ogen) degradation products (FDPs)^{4,5}. Besides the commonly investigated breakdown products X, Y, D and E, plasmin liberates several small peptides from human fibrinogen designated "micromolecular FDPs"⁶. These are dialysable, thermostable, have molecular weights between 15,000 and 500 (ref. 6) and have distinct physiological properties⁷⁻¹⁰. Since both the degree of impairment of lymphocyte function and the frequency of FDP formation are related to the extent of neoplastic spread^{3,4}, we investigated the influence of micromolecular FDPs on cellular and humoral immune responses. A striking immunosuppressive activity was found, possibly responsible for impaired immunoresponsiveness in cancer patients. Terminal FDPs were obtained by prolonged digestion of human fibrinogen (Forschungsfibrinogen Kabi) with human plasmin (Forschungsplasmin Kabi) in aqueous solution, pH 7.4, and dialysed against distilled water at 4 °C for 24 h. The dialysate was concentrated by evaporation in a vacuum, reconstituted in physiological saline, sterilised by filtration and added to lymphocyte cultures using a microculture system¹¹. As Fig. 1 shows, micromolecular FDPs caused a dose-dependent suppression of PHA-induced lymphocyte transformation, which was 98% at 1.1 mg ml⁻¹. These concentrations did not affect cell viability as measured by Trypan blue exclusion. Plasmin dialysates alone, prepared in identical conditions, inhibited neither of the systems tested: separation of fibrinogen on Sephadex G-200 showed no evidence of pre-existing contaminants (Fig. 4).

To characterise the active components, micromolecular FDPs were separated on Sephadex G-25. All preparations revealed four peaks (1, 2, 3 and 4), the first appearing with the void volume (Fig. 2). Peaks were reduced to the initial volume of the sample and tested in the lymphocyte culture. A marked inhibitory activity was always found in peak 3; peaks 1 and 4 were slightly suppressive.

Additional experiments were designed to test the effect of micromolecular FDP on sheep red blood cell (SRBC)-specific primary immune responses in Swiss mice. Animals

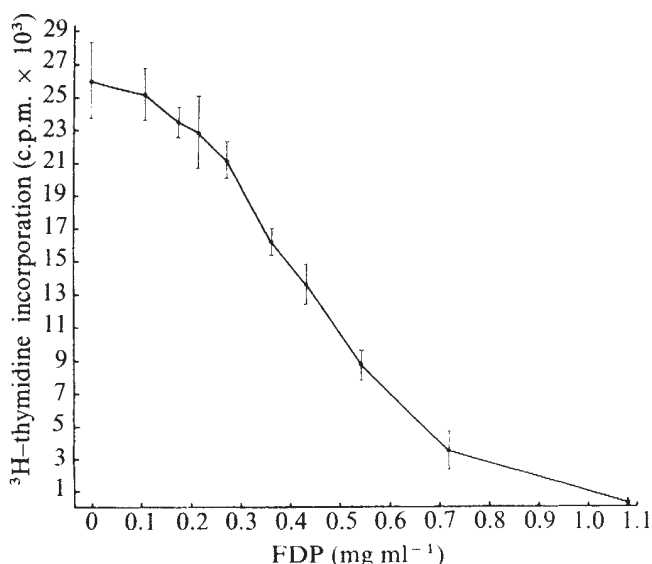


Fig. 1 Dose-dependent suppression of PHA-induced ³H-thymidine incorporation of human peripheral lymphocytes by increasing amounts of micromolecular FDPs. Protein concentrations were assayed according to Lowry *et al.*¹⁹. After purification on Ficoll-Hypaque 200,000 cells were cultured in 0.1 ml of Eagle's MEM with 10% pool human AB serum using 0.1 ml of PHA-P (Difco) as a stimulant (25 µg per ml culture fluid). Samples of 20 µl of FDP dilutions in saline were added; controls included saline instead of FDPs and MEM instead of PHA. Cultures were collected after 66 h.

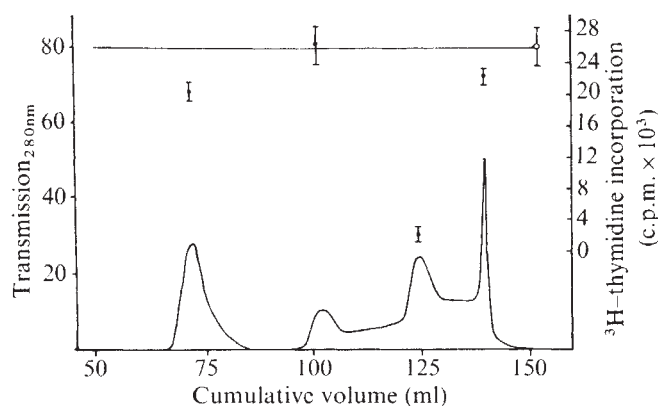


Fig. 2 Inhibitory activity of micromolecular FDPs separated by Sephadex G-25 gel filtration on PHA-induced lymphocyte transformation. Chromatography was carried out using column K 16 (Pharmacia, void volume 65 ml) and distilled water as eluant. Peaks were concentrated by evaporation in a vacuum, reconstituted with saline in the original sample volume, and tested as described in Fig. 1. ○, c.p.m. of controls with saline; ●, c.p.m. of cell cultures with FDPs added (mean $\pm$ s.d.).

were immunised intraperitoneally with 4×10^8 SRBCs and simultaneously injected intravenously with various dilutions of FDP. The splenic content of SRBC-specific plaque-forming cells (anti-SRBC PFC) was determined according to Jerne and Nordin¹². The mean number of anti-SRBC PFC per 10^6 spleen cells in primed controls was 1,820 (Fig. 3). Unprimed controls either treated with FDP ($n=3$) or untreated ($n=6$) produced only few "background-plaques" (6–12 PFC per 10^6 cells). Treatment with 100 μ g of FDPs did not alter significantly the SRBC-specific immune response, whereas administration of 130–160 μ g markedly inhibited PFC production (mean value of 998 PFC per 10^6 cells).

To test the effect of different timing on specific PFC production, this dose was administered at different times before and after primary immunisation. The most striking immunosuppression (about 80%) occurred in mice treated with 130–160 μ g of FDPs 24 h after priming, whereas PFC responses in animals treated with 100 μ g of FDPs were not significantly different from the control value (Fig. 3).

These findings clearly demonstrate a time-dependent and dose-dependent immunosuppressive action of micromolecular FDP. In this experimental model FDPs act predominantly in the early phase of clonal proliferation, that is 24 h after antigenic challenge. By this time, DNA synthesis will be activated, but significant antibody production is still lacking.

Since we suspected a relationship between immunosuppressive activity and circulating FDPs in the blood of cancer patients, we studied 19 donors with malignant diseases and three patients with hepatic cirrhosis to look

for a correlation between both findings. Because direct measurements of micromolecular FDP are not yet available, we determined the higher molecular weight FDPs in plasma by means of reptilase time, thrombin coagulase time and the ethanol gel test, and in serum using the Laurell technique after collecting blood with Aprotinin.

Serum samples were fractionated by Sephadex G-200 chromatography, and the fourth peak containing the peptide material¹ (Fig. 4) was further chromatographed on Sephadex G-25 as described for FDP. Chromatography of some sera revealed a first peak (A) corresponding to peak 1 of FDP; in all sera except one, two further peaks appeared, one of which (B) corresponded to peak 3, the other (C)

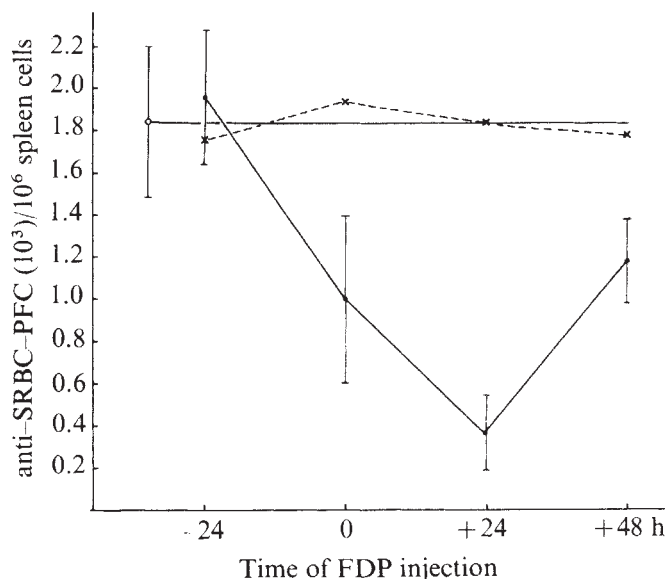


Fig. 3 Dose-dependent and time-dependent suppression of splenic production of anti-SRBC PFC by micromolecular FDPs in Swiss mice. FDP dilutions were administered intravenously in a single injection at the intervals indicated with respect to intraperitoneal primary immunisation with 4×10^8 SRBC. Data represent day-4 PFC responses per 10^6 spleen cells (vertical bars indicate s.d.) calculated for groups of six mice. Primed animals receiving no further treatment served as controls ($n=22$). ○, Controls; ●, 130–160 μ g FDP; ×, 100 μ g FDP.

to peak 4 of micromolecular FDPs. In the peak 2 region of FDPs no material appeared. Peaks and sera were again added to lymphocyte cultures, and a significant immunosuppressive activity was defined as at least 40% inhibition of PHA-induced 3 H-thymidine incorporation.

Representative experiments are shown in Table 1. The sera and/or Sephadex G-25 peaks of seven patients without signs of circulating FDPs (cases 1–7) revealed no immunosuppressive activity, whereas those of eleven patients with demonstrable FDP (cases 8–18) inhibited lymphocyte transformation markedly. In only four cases investigated so far

Table 1 Occurrence of FDP and of inhibitory plasmatic activity (representative cases)

Case	Reptilase time (s)	Thrombin coag. time (s)	Ethanol gel test	FDP Laurell (mg%)	% Inhibition by serum or peaks of rechromatography			
					Serum	peak A	peak B	peak C
2	14.3	18.1	Neg.	0	15	—	—	28.5
5	11.5	16.8	Neg.	0	24.9	—	0	0
6	17.9	18.9	Neg.	0	32.6	—	32.4	32.6
12	27.3	26.0	Pos.	10.0	45.4	—	71.3	16.0
16	32.3	30.9	Pos.	19.0	68.5	—	99.6	68.4
18	21.4	19.8	N.D.	25.0	N.D.	—	98.6	11.9

Diagnosis of all patients investigated: Chronic lymphocytic leukaemia (1,4), anaplastic Ca unknown origin (2), chronic uraemia with Ca rectum (3) Ca kidney (5,14), Hodgkin's disease (6,10,15,20), metastatic Ca mamma (7), liver cirrhosis (8,9,21), metastatic adeno-Ca unknown origin (11), malignoma suspected (12), metastatic solid Ca unknown origin (13), Ca pancreas (16,17), Ca bronchus (18,19), adeno-Ca stomach (22). —, No peak eluted in this position. N.D., not done. For normals reptilase time and thrombin coagulase time were 22–24 s; ethanol gel test was negative and FDP Laurell was not measurable.

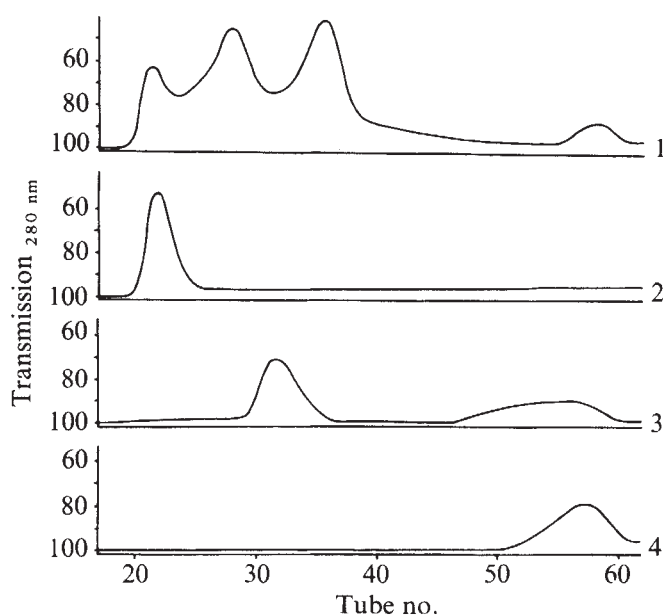


Fig. 4 Elution patterns (Sephadex G-200) of a patient's serum (curve 1), fibrinogen Kabi before digestion (curve 2), plasmin-digested fibrinogen (curve 3), and the micromolecular FDP separated by dialysis (curve 4). Column K 16 (Pharmacia), 0.1 M Tris-buffer, pH 7.4 as eluant. The micromolecular immunosuppressive molecules appear during plasmin digestion of fibrinogen, the dialysable moiety of which is eluted in the identical position as serum peak 4.

(19–22) was no direct correlation between inhibitory serum factors and circulating FDP evident. The inhibitory activity was most frequently localised in peak B, corresponding to the maximal inhibitory region of FDP eluates. Certain cases—especially with evidence of high FDP values in plasma—showed additional or predominant inhibition in peak C.

Our preliminary results suggest that micromolecular FDP, which are known to inhibit thrombocyte aggregation^{7,9,10} and to influence the tonus of smooth muscles⁸, also have immunosuppressive activity. According to separation characteristics on Sephadex G-25, their molecular weight ranges below 5,000. Whether these peptides are identical with other serum inhibitors, not yet characterised definitively, needs further investigation. Clearly, however, in most conditions for which the presence of immunosuppressive serum factors has been reported, fibrinogen degradation products do indeed occur—in carcinomas^{2,4}, Hodgkin's diseases^{3,13}, renal diseases^{14,15}, pregnancy^{3,16} and transplant rejection^{17,18}. Furthermore, it seems remarkable that in two of our cases with a non-neoplastic disease (hepatic cirrhosis) FDP-containing sera markedly inhibited lymphocyte transformation.

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Correlation between specific cytotoxicity and expression of idiotype receptors of allograft-infiltrating cells

REJECTION of an allograft is normally accompanied by infiltration of the graft by host cells. The origin, type and receptor specificity of these cells as well as their precise functions are largely unknown, primarily because of the extreme difficulties of quantitative recovery of the infiltrating cells. A promising experimental technique¹ has recently been developed: a spongy matrix tissue is infiltrated by strain A fibroblasts, transplanted into B animals and removed at various times thereafter. Mere physical compression of the 'sponge' releases virtually all of the infiltrating B cells whereas virtually none of the A fibroblasts will be released. The released B-strain cells are functionally intact.

It has been found possible to visualise and enumerate lymphoid cells with specific immune reactivity toward major histocompatibility locus-determined antigens^{2,3}. Such visualisation is achieved by the use of specific anti-idiotypic antibodies selectively reacting with B and T lymphocytes carrying idiotype receptors signifying reactivity against a certain alloantigen. We here describe the cytolytic capacity and idiomorph expression of cells invading an *in vivo* sponge 'allograft'.

Fibroblasts were allowed to invade sponge matrix allografts by implantation into the peritoneal cavities of DA, Lewis or BN rats. These strains differ at the major histocompatibility locus⁴. Five days later the sponges were removed and transplanted subcutaneously into the neck of Lewis or DA rats. These rats were killed seven or eight days later (at peak killer activity in the graft (P. J. Roberts and P.H., unpublished)) and the sponges, as well as the draining (axillary-inguinal) and non-draining (popliteal) lymph nodes and the spleens were removed. Cell suspensions were prepared as described previously.

Table 1 Expression of anti-DA idiotype receptors on graft-infiltrating cells, on draining and non-draining lymph node cells and on spleen cells seven days after grafting of a syngeneic or allogeneic sponge matrix graft

	Graft	Source of cells* Draining lymph nodes	Non-draining lymph nodes	Spleen
Lewis carrying DA graft	56.0	25.1	4.5	9.1
DA carrying Lewis graft	1.3	0.7	0.0	0.1
Lewis carrying Lewis graft	2.0	5.8	5.0	4.9
Lewis carrying BN graft	3.9	7.5	5.4	5.7
Normal Lewis rat	ND	ND	4.5	5.2
Normal DA rat	ND	ND	0.1	0.2

*Figures refer to the percentages of cells stained by direct immunofluorescence with FITC-conjugated anti-(anti-DA) antiserum³. ND, not done.

The percentages of cells displaying idiotype receptors of Lewis-anti-DA specificity in the various cell-suspensions were analysed using FITC-conjugated anti-Lewis-anti-DA IgG antibodies. As seen in Table 1, low 'background noise' staining was observed in the control DA rats (highest percentage 'positive' cells being 1.3% in the most macrophage-rich population). In contrast, the Lewis cells from normal rats, or from rats carrying BN grafts, were all within the previously found range of percentage³ (2–7.5% idiotype positive cells). In Lewis cell populations from rats receiving DA sponges, a normal (4.5% anti-DA) idiotype frequency in the non-draining nodes, a slight increase in the spleen, and highly significant increases in the draining node cells and particularly among the sponge-invading cells were recorded. Thus, a highly selective increase of cells expressing idiotypes signifying anti-DA reactivity were indeed found infiltrating DA grafts transplanted into Lewis rats. Two similar experiments yielded essentially similar results, with a variation in the data of $\leq 20\%$.

In a second series of experiments cells invading a sponge allograft (and cells from the various lymphoid organs) were

analysed for idiotype-positive cells, as well as for specific cytolytic activity *in vitro* against relevant target cells. To further define the participating effector cells in this system, an investigation was carried out to determine the percentage of phagocytic or IgG-coated, latex positive (bound on the surface) cells among the idiotype-positive and negative cells. The results of such experiments are shown in Table 2. A clearcut positive correlation between the occurrence of an increased percentage of cells with anti-DA idiotypes and specific anti-DA cytolytic capacity of the same cell suspension was observed (see the results obtained using cells from Lewis rats grafted with DA sponges). When the capacity to bind latex particles on the cell surface and/or phagocytic activity were investigated, striking differences were observed between idiotype-positive cell populations obtained from the various rats and organs.

Lewis-anti-DA idiotype-positive cells obtained from lymph nodes or spleens always contained a majority of latex negative cells irrespective of whether the Lewis rats had, or had not, received any BN or DA sponges. This is to be expected since Lewis-anti-DA idiotype-positive T cells

Table 2 Percentage anti-DA idiotype carrying cells and cytolytic anti-DA activity seven days after grafting of syngeneic or allogeneic sponges. Distribution of receptors for Fc of Ig and phagocytosis in idiotype-positive and negative cells

Rat	Source of cells Organ	Total anti-DA idiotype positive* (%)	Percentage distribution of latex-positive and phagocytic† cells within the idiotype-positive or negative cells				Cytolytic‡ anti-DA activity
			Expression of idiotype	Latex negative	Latex positive on cell surface	Latex positive phagocytosing	
Lewis carrying DA graft	Graft	42.8	pos.	51.2	7.2	41.6	44.8
			neg.	49.1	3.2	47.8	
	Draining lymph node	21.1	pos.	72.0	13.0	13.0	22.5
			neg.	96.0	2.0	1.0	
	Non-draining lymph node	5.2	pos.	92.3	1.9	5.8	4.8
			neg.	88.8	10.6	0.7	
DA carrying Lewis graft	Spleen	9.2	pos.	72.8	21.7	5.4	26.0
			neg.	58.3	37.3	4.4	
	Graft	1.3	pos.	0.0	7.7	92.3	ND
			neg.	56.1	2.1	41.7	
	Draining lymph node	0.7	pos.	0.0	0.0	100.0	ND
			neg.	80.4	9.4	10.3	
Lewis carrying Lewis graft	Non-draining lymph node	0.0	pos.	ND	ND	ND	ND
			neg.	88.4	11.3	0.3	
	Spleen	0.1	pos.	0.0	0.0	100.0	ND
			neg.	64.4	31.3	4.3	
	Graft	2.0	pos.	60.0	40.0	0.0	–0.9
			neg.	50.7	3.0	46.2	
Lewis carrying BN graft	Draining lymph node	5.8	pos.	86.2	12.1	1.7	1.2
			neg.	79.2	9.9	10.9	
	Non-draining lymph node	5.0	pos.	86.0	14.0	0.0	ND
			neg.	85.5	14.1	0.4	
	Spleen	4.9	pos.	67.4	18.4	14.3	0.4
			neg.	63.0	33.6	3.4	
Lewis carrying BN graft	Graft	3.9	pos.	43.6	18.0	38.5	5.3
			neg.	49.1	4.5	46.4	
	Draining lymph node	7.5	pos.	80.0	6.7	13.3	3.4
			neg.	81.3	8.6	10.1	
	Non-draining lymph node	5.4	pos.	90.8	7.4	1.8	2.2
			neg.	87.4	11.3	1.3	
Normal Lewis rat	Spleen	5.7	pos.	73.7	17.5	8.8	1.3
			neg.	61.3	33.6	5.1	
	Lymph node	4.5	pos.	91.1	6.7	2.2	ND
			neg.	87.5	10.8	1.7	
Normal DA rat	Spleen	5.2	pos.	71.8	19.3	9.6	ND
			neg.	63.5	32.3	4.1	
Normal DA rat	Lymph node	0.1	pos.	0.0	0.0	100.0	ND
			neg.	85.6	13.7	0.7	
	Spleen	0.2	pos.	0.0	0.0	100.0	ND
			neg.	63.8	31.4	4.8	

*Total number of anti-Id carrying cells in a given sample by direct immunofluorescence with FITC-conjugated anti-(anti-DA) antiserum.

†The cells were first incubated at 37 °C for 30 min with IgG-coated latex particles. After preparation of the cell smears, each cell was investigated for the presence/absence of surface immunofluorescence, for the presence/absence of latex particles attached on to the cell surface ('Fc-receptor' carrying cells) and for latex particles ingested inside the cell (phagocytic cells). All cells phagocytosing latex particles also attached latex particles on their surface.

‡The killer assay was run overnight in V-shaped microtitre plates using a killer–target cell ratio of 100:1 using ⁵¹Cr-labelled PHA blasts as target cells, with the media and killer conditioned essentially as described before³.

normally outnumber B cells carrying the same idiotype by a factor of 5–7 (ref. 3). Graft-invasive idiotype-negative cells had, on average, a higher percentage of latex-positive, phagocytic cells than cell suspensions obtained from lymph nodes or spleens. The 'background' noise of false 'positive' anti-DA idiotype cells in the fluorescent antibody technique was caused by latex-positive, phagocytic cells (see, for instance, figures obtained using cells from DA rats receiving Lewis sponge). We interpret this false positive staining to arise from complexed FITC-IgG molecules being bound by way of Fc receptors. In the DA grafts transplanted into Lewis rats, however, a very high percentage of the total population of graft-invasive cells displayed anti-DA idiotypes as well as the capacity to bind latex particles on the cell surface and phagocytic activity (compare with composition of cells extracted from BN sponges). The exact nature of these latter idiotype-positive, latex-binding and phagocytic cells is unknown, but they may very likely be of macrophage origin. If so, the presence of idiotype-positive material on the surface of the DA sponge invading macrophages would still be obscure (whether from specific T cells⁶ or from surface binding of complexes made up of Lewis-anti-DA alloantibodies and DA alloantigen). Also, it would remain to be established whether such idiotype-coated macrophages could act like specifically armed macrophages with regard to DA target cells. Fractionation of the various cell types with subsequent analysis for cytotoxic activity are under way to determine these questions.

In conclusion we can state that an allograft is invaded by a complex variety of cells carrying actively produced or passively adsorbed idiotype receptors on the surface with specific reactivity for the target alloantigens. A positive correlation could be found between the presence of increased numbers of such idiotype-positive cells and the specific cytolytic activity against the relevant alloantigen.

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Cytotoxicity of antibody-coated trypanosomes by normal human lymphoid cells

DESTRUCTION of antibody-coated mammalian target cells by normal human lymphocytes demonstrated by release of ⁵¹Cr-chromate is a phenomenon characterised by its rapidity of action and by the very small amounts of antibody required. It seems unlikely that such a mechanism destroys solely mammalian target cells, and possibly it has a role in immunity to parasitic infections. Butterworth *et al.*¹ demonstrated that normal human blood cells destroy

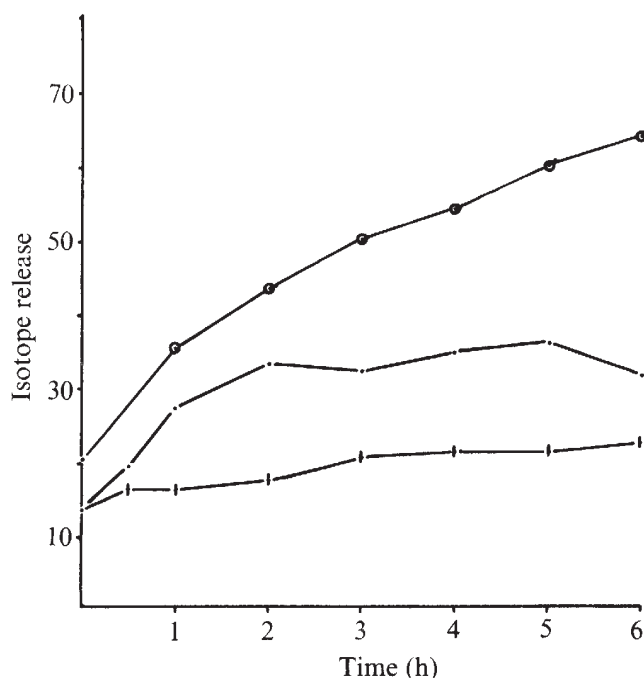


Fig. 1 Time course of isotope release from *T. dionisii* culture forms. Circle and dot, isotope release from parasites incubated in the presence of antibody (1/100 dilution) and normal human lymphocytes; dot, parasites incubated in the presence of normal human lymphocytes alone; vertical line, incubated in medium alone. Statistical summary: analysis of variance—interaction of time with treatment (antibody, lymphocytes or medium) very significant ($P < 0.05\%$; $F = 5.5$, d.f. 14 and 72; limiting value for $F = 3.82$). Log_e least significant differences for comparison of means up to 0.239, corresponding to a difference of 5.3% at 20% isotope release, 10.7% at 40% and 16% at 60%.

schistosomula coated with antibody from infected patients.

We present here evidence that normal human lymphoid cells in the presence of specific antibody can cause isotope release from culture forms of *Trypanosoma* (*Schizotrypanum*) *dionisii*, a parasite related to *T. cruzi* with an intracellular stage in its vertebrate host (bats).

Protozoa do not take up enough ⁵¹Cr-chromate to make it possible to use this label in cytotoxicity experiments. Technetium (as ^{99m}Tc-pertechnetate) can be used as a label for target cells in cytotoxicity experiments if non-radioactive sodium chromate is present in the labelling mixture (unpublished results of D.F., B. Tayabali and P. O'Brien); the target cells are presumably labelled with some complex organometallic ion of chromium and technetium. The isotope is released when the cells are killed, is not reutilised and, like ⁵¹Cr-chromate, gradually leaks out of target cells. Repeated freezing and thawing causes the release of about 70 or 80% of the isotope, and treatment of labelled protozoa with detergent Nonidet causes the release of all the isotope. In all respects, therefore, protozoa labelled with technetium in this way behave like mammalian cells labelled with either ⁵¹Cr-chromate or ^{99m}Tc-pertechnetate.

Culture forms of *T. dionisii* were grown in a liquid medium (L4N (ref. 2) with the concentration of serum and erythrocyte lysate reduced by half). They were washed three times and incubated with about 1 mCi of sterile ^{99m}Tc-pertechnetate eluted from a generator (Radiochemical Centre). To this mixture was added 0.1 ml of a solution of sodium chromate ($13.5 \mu\text{g ml}^{-1}$ in phosphate-buffered saline). After incubation at 37 °C for 1 h, the organisms were washed repeatedly (usually five times) until the count rate in the supernatant had reached an acceptable level (less than 5% of the label in the protozoa). The organisms were then made up to 10⁵ per ml. Tissue culture Medium

Table 1 Isotope release from ^{99m}Tc -pertechnetate labelled *T. dionisii* in the presence of antibody and normal lymphoid cells

Experiment	Time (h)	Dilution of antibody			Lymphocytes alone	Medium alone
		1/10	1/100	1/1,000		
1	3	70.6	61.7	59.9	26.7	17.0
	6	76.9	73.9	67.5	35.0	17.0
2	3	59.7	59.9	48.8	17.8	12.3
	6	72.5	60.0	58.5	16.5	16.3
3	3	47.6	—	26.5	19.8	12.2
	6	49.9	—	26.8	18.2	17.0

Controls: normal rabbit serum alone and antiserum to *T. dionisii* alone gave isotope release not significantly different from that in the presence of medium. Normal rabbit serum with lymphoid cells gave isotope release not significantly different from that of lymphoid cells alone.

Summary of statistical analysis. Effect of antibody treatment very highly significant in all experiments ($P < 0.05\%$). Experiments 1 and 2: $F = 469$, d.f. 4 and 90; limiting value for $F = 5.6$; experiment 3: $F = 83$, d.f. 3 and 54; limiting value for $F = 7.01$. Log_e least significant difference for comparison of means up to 0.128 for experiment 1 (corresponding to differences of 4% at 30% isotope release and 8.2% at 60%), up to 0.164 for experiment 2 (corresponding to 5.3% at 30% and 10.6% at 60%) and up to 0.174 for experiment 3 (corresponding to 3.8% at 20% and 9.5% at 50%).

199 with 10% foetal calf serum (previously heated at 56 °C for 2 h) was used throughout. Normal human peripheral blood lymphoid cells were prepared by the methocel/carbonyl iron method from defibrinated blood. Rabbit antiserum to *T. dionisii* prepared by three injections (10^7 organisms in Freund's complete adjuvant intramuscularly, subcutaneously; and 10^7 sonicated organisms intravenously) at 14-d intervals, and collected 12 d after the last injection, was heated to 56 °C to destroy complement activity, and diluted in medium. Samples of 100 μl of labelled *T. dionisii* (10^4 organisms), 100 μl of diluted rabbit antiserum and 100 μl of human lymphoid cells (2×10^5 cells) were mixed in small plastic tubes and incubated at 37 °C for up to 6 h. After this time half of the supernatant was removed from the tube and counted in a sodium iodide crystal scintillation counter together with the tube containing the remainder of the supernatant and the remaining organisms, so that the percentage of isotope released could be calculated. All experiments were subjected to statistical analysis by analysis of variance, and differences between means of groups of tubes were tested for significance by Duncan's multiple range test.

Table 1 shows that normal human lymphocytes in the presence of dilutions of antibody as high as 1/1,000 cause considerable isotope release from the parasites. Lymphocytes alone cause more isotope release than the spontaneous leakage from organisms incubated in medium as also occurs when human target cells are incubated in the presence of human lymphoid cells, and its significance is not understood.

Figure 1 shows that significant isotope release occurs within 1 h and progresses to completion at about 6 h. Isotope release in the presence of lymphocytes alone also increases, though more slowly. Spontaneous leakage of isotope from organisms incubated in medium alone remains fairly constant between 1 and 6 h.

It would clearly be premature to claim that this demonstration of antibody-dependent lymphocyte-mediated cytotoxicity of *T. dionisii* establishes its role in immunity against protozoal parasites. Nevertheless, the requirements for very small amounts of antibody, its dependence on a ubiquitous normal cell, and the rapidity of its action at least make this form of cytotoxicity a candidate which may have an important role in limiting the spread of intracellular parasites from one cell to another.

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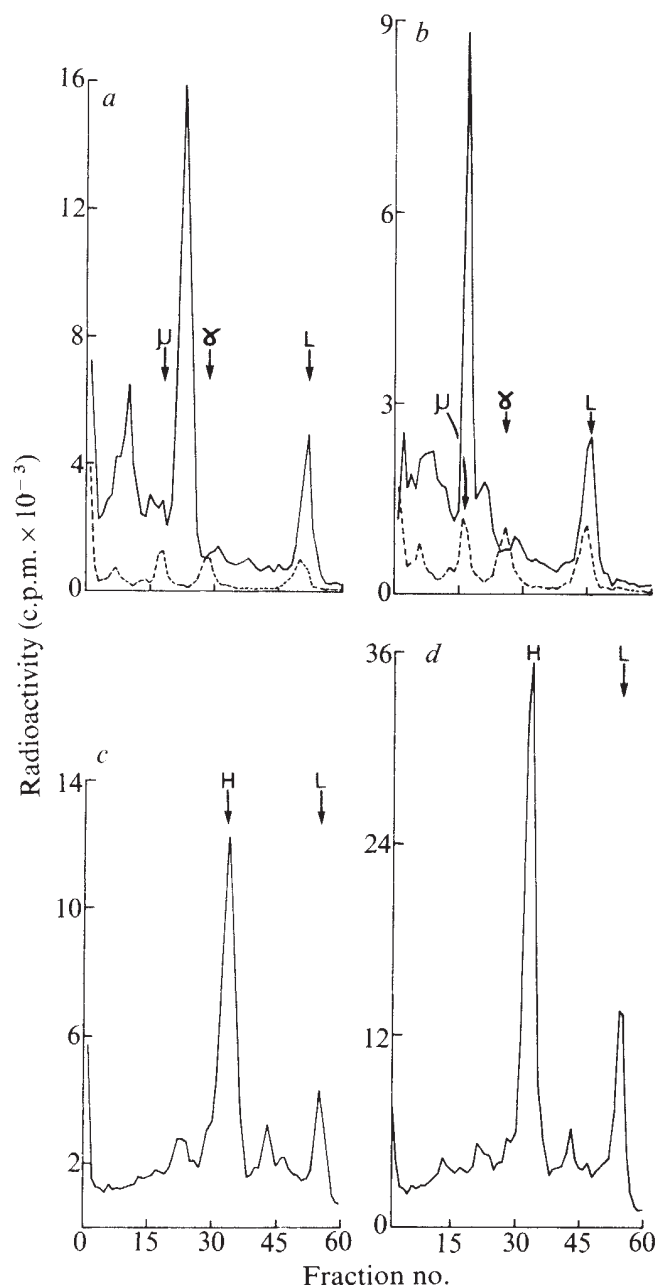
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Preparation and characterisation of an antiserum to the mouse candidate for immunoglobulin D

ANALYSIS of mouse B-lymphocyte surface immunoglobulin (Ig) by isotopic labelling techniques reveals little, if any, IgA or IgG. Instead, the surface Ig consists of monomeric IgM and an Ig similar in physicochemical properties to human IgD¹⁻³. In the absence of sequence comparison between human IgD and its assumed mouse counterpart, this assignment is tentative. With this proviso, however, and for the sake of clarity in presentation, we refer to this immunoglobulin as IgD. During development, IgM-bearing cells are the first to arise and the ratio of IgM-IgD determined with labelled adult lymphocytes is higher in the spleen than in lymph nodes or Peyer's patches¹⁻⁴. These findings, however, give the total yield of the two Ig classes and not their distribution on individual lymphocytes. On the basis of the absence of immunoglobulins other than IgM or IgD on the surface of B lymphocytes, we indicated the presence of cells bearing IgM or IgD, or IgM and IgD in mouse spleen cell suspensions^{5,6}. In these experiments, IgM was first capped with rhodamine-labelled anti- μ and subsequent ring staining with fluorescein-labelled anti-Fab in the presence of sodium azide was taken as evidence for the presence of IgD. We report here the preparation of an antibody specific for mouse IgD, and its use in the characterisation of mouse B-lymphocyte surface Ig.

Plasma cell membranes were prepared from the spleens of 1,750 BALB/c mice (wet weight 160 g) to yield 130 mg of protein. The membranes were dissolved in 25 ml of phosphate-buffered saline (PBS) containing 1% (w/v) Nonidet P-40 and 1 mM phenylmethylsulphonyl fluoride, centrifuged at 10⁵g for 3 h, and then passed successively through three columns of Sepharose 4B: (1) normal rabbit Ig (6.0 mg)-Sepharose (3.0 ml); (2) rabbit anti-mouse μ (3.6 mg)-Sepharose (1.8 ml); (3) rabbit anti-mouse Fab (5 mg)-Sepharose (2.5 ml). The rabbit anti-mouse μ was prepared by applying a specific rabbit anti- μ serum to IgM (λ^1) (MOPC 104E)-Sepharose, and then eluting the antibody with molar acetic acid. Anti-Fab was prepared similarly and was a mixture of the following acid eluates: rabbit anti-whole IgG₁(K)(MPC 21) retained by IgG_{2a}(K) (Adj PC5)-Sepharose, rabbit anti-whole IgG_{2a}(K)(Adj PC5) retained by IgG₁(K)(MPC 21)-Sepharose and rabbit anti-whole IgM(K)(TEPC 183) retained by IgG_{2a}(K)(Adj PC5)-Sepharose. Purified antibody was coupled to Sepharose⁸ to give high capacity immunoabsorbents. After passage of the sample, the columns were washed with phosphate-buffered



saline (PBS), emulsified in complete Freund's adjuvant and injected into rabbits⁹. Each column was divided between two rabbits. Serum from a rabbit receiving the third column, the anti-Fab, was passed over IgG_{2a}(K)(Adj PC5)-Sephadex and then IgM(K)(TEPC 183)-Sephadex. The absorbed serum was tested by radioimmunoassay⁵ and found to be unreactive with ¹²⁵I-labelled IgM(K)(TEPC 183), IgM(λ_1)(MOPC 104E), IgA(λ_2)(MOPC 315), IgG₁(K)(MPC 21), IgG_{2a}(K)(Adj PC5), IgG_{2b}(K)(MOPC 195), IgG₃(FLOPC 21), λ chains (RPC 20), K chain (MOPC 41) and mouse α_2 -macroglobulin. The radioimmunoassay will detect less than 1 μ g of antibody in 1 ml of serum.

When used in conjunction with rhodamine-labelled goat anti-rabbit Ig (ref. 10), the absorbed antiserum stained¹¹ about 20% of CBA strain spleen cells, but not thymocytes from CBA, C57BL, DBA/2, C3H, AKR and BALB/c mice. By this test, the antiserum was also negative for peripheral T cells prepared by the nylon wool method¹².

We then evaluated the antiserum by testing with ¹²⁵I-surface labelled spleen cells as described before¹, and found the only Ig precipitated corresponded to IgD (Fig. 1a, refs

Fig. 1 Surface immunoglobulin of murine splenic lymphocytes. Spleen cell suspensions of 3-6-month-old, specific pathogen-free, female CBA mice were labelled with ¹²⁵I by the lactoperoxidase-catalysed procedure¹⁸, washed once in ice-cold PBS and then lysed for 10 min at 0 °C in 1% (w/v) Nonidet P-40 in PBS containing 1 mM phenylmethylsulphonyl fluoride and 100 mM recrystallised iodoacetamide. The centrifuged lysate (30,000g $\times$ 15 min) was passed over Sephadex G-25 equilibrated with the solution used for lysis of the cells. Labelled IgD was precipitated at 0 °C by addition of rabbit antibody (10 μ l) and a goat anti-rabbit Ig (100 μ l). The precipitate was removed by precipitation and the supernatant was further reacted with rabbit anti-mouse μ chain (10 μ l) and goat anti-rabbit IgG (100 μ l). Both precipitates were washed three times with ice cold 0.5% (w/v) Nonidet P-40 in PBS, once with 50 mM sodium phosphate, pH 7.0, and then dissolved in 50 mM sodium phosphate, pH 7.0, containing 2% (w/v) sodium dodecyl sulphate (SDS) and 1 mM dithiothreitol. The precipitates were heated at 100 °C for 10 min, and then iodoacetamide was added to 100 mM. Internal ¹³¹I-labelled¹⁹ markers were added to the labelled cell surface Ig samples, which were then resolved by SDS-polyacrylamide gel electrophoresis¹. After electrophoresis the gels were sliced into 1-mm segments and radioactivity was determined. Values were corrected for cross-channel spill and plotted with the top of the gel on the left hand side of the figure. Reduced cell surface Ig precipitated with the anti-IgD (a and c) and subsequently anti- μ (b and d) were electrophoresed on 7.5% (a and b) and 4.2% (c and d) polyacrylamide gels. —, ¹²⁵I incorporated into cell surface Ig; . . . , ¹³¹I-labelled internal marker.

1-4). A similar profile resulted when 100 μ l of normal mouse serum was incubated with 10 μ l of the rabbit antiserum (30 min room temperature) before addition of the radioactive sample. Thus circulating IgD is likely to be present in very small amounts in the mouse. Recovery of IgM was possible from the supernatant of the first precipitation by addition of anti- μ (Fig. 1b). The radioactive material which runs before and after the μ and δ chains also occurs in non-specific precipitates. Furthermore, the higher molecular weight material largely disappears when less concentrated polyacrylamide gels are used (Fig. 1c and d), and thus may represent an aggregation artefact. The disadvantage of the less concentrated polyacrylamide gels is that there is no resolution between the μ and δ chains. When unreduced samples of cell surface Ig were electrophoresed on 4.2% (w/v) polyacrylamide gels, the material then coincided with a disulphide-linked 7S IgM internal marker¹.

On the basis of these results, we were therefore confident that our antiserum was specific for mouse IgD, and thus turned to studies using fluorescent reagents prepared by DEAE chromatography¹⁰ to stain live cells¹¹. With 1-2-week-old CBA mouse splenocytes, we were unable to find significant numbers of cells reacting with the anti-IgD, whereas anti-mouse μ chain revealed 8-14% of the cells as positive. Cells bearing IgD were, however, readily apparent in the spleens of 6-week-old mice (~20% total lymphocytes). Absorption of the antiserum with foetal liver cells (3.6×10^{10} cells per ml of serum) did not remove its capacity to react with spleen cells, whereas absorption with adult spleen cells did (1.9×10^{10} cells per ml of serum). The finding that IgM precedes IgD in ontogeny^{1,3} is therefore confirmed.

We next determined the distribution of IgM and IgD on lymphocytes from various lymphoid organs (Table 1). Single staining was done by the indirect procedure. Double staining was achieved by capping IgD with a fluorescein-labelled system followed by staining for IgM with a rhodamine-labelled rabbit anti-mouse μ chain under non-capping conditions. From the single staining results it is immediately clear that IgD constitutes the major cell surface immunoglobulin of lymph nodes and Peyer's patches, as would be suggested by biochemical investigations³⁻⁴. Double staining of lymphocytes from these organs not surprisingly revealed most of the Ig-positive cells as having only IgD, although some doubles (IgM and IgD) and occasional cells positive for IgM alone were also present. In the spleen, however,

Table 1 Frequency of IgM and IgD-bearing cells in mouse lymphoid tissue

	Organ	Anti-Ig	Single staining		Double staining	
			Anti- μ	Anti- δ	$\mu + \delta$	δ
Experiment 1	Peripheral nodes	14.9	3.1	10.5	0.7	1.5
	Mesenteric nodes	14.4	4.1	10.5	1.1	1.8
	Peyer's patches	23.1	8.5	26.8	1.6	8.5
	Spleen	35.6	19.1	23.8	12.1	13.8
Experiment 2	Peripheral nodes	15.6	3.1	9.1	0.5	2.7
	Mesenteric nodes	14.7	3.6	12.6	0.8	2.8
	Peyer's patches	23.2	9.4	22.5	0.8	8.2
	Spleen	32.2	17.8	25.9	8.7	11.9

Cell suspensions were prepared from 7-month-old, specific pathogen-free, female CBA mice by teasing the organs with veronal-buffered saline (Oxoid), containing bovine serum albumin (1 mg ml^{-1}) (VBS-BSA), and the living cells were stained and prepared for examination as described by Raff¹¹. Peripheral nodes collected were superficial cervicals, axillary, brachial and inguinal. For single staining the cells were incubated for 20 min at room temperature with rabbit antiserum, washed three times with VBS-BSA and then suspended in rhodamine-labelled goat anti-rabbit IgG (20 min, room temperature). Samples were washed, and then mounted in 50% (w/v) glycerol in (PBS)-0.03 M sodium azide. For double staining, cells were treated with anti-mouse IgD and fluorescein-labelled goat anti-rabbit IgG as described above. The two layers were essential to ensure capping of all the IgD positive cells. The cells were then incubated in the cold for 20 min with rhodamine-labelled rabbit anti-mouse μ chain and with sodium azide at 0.03 M. After washing with cold VBS-BSA-0.03 M sodium azide the cells were mounted and examined under ultraviolet light with a Leitz Orthoplan research microscope fitted with Ploem illumination. At least 500 lymphocytes were assessed for staining and the results are given as percentages of total lymphoid cells observed. All reagents were centrifuged (50,000g, 60 min) on the day of use. On the basis of the double staining protocol, lymphocytes could be classified into three groups, those with green caps (IgD positive), those with peripheral red staining (IgM positive) and those with green caps and red rings (IgM and IgD positive). The rabbit anti-mouse Ig was prepared by immunisation of rabbits with the Fab portion of myeloma protein Adj PC5 (γ_{2a} K) (ref. 20) as described before⁹ and was polyspecific, reacting against IgM, IgG₁, IgG_{2a}, IgG_{2b}, IgG₃ and IgA (but not other serum proteins) on Ouchterlony analysis. Rabbit anti-mouse μ chain was prepared by immunisation with myeloma protein MOPC 104E ($\mu\lambda_1$)⁹ and absorbing the serum with a 7S fraction of normal mouse serum coupled to Sepharose 4-B. The resulting serum precipitated IgM on Ouchterlony analysis, but did not react with any other mouse Ig class when tested by radioimmunoassay against ¹²⁵I-labelled immunoglobulins (see the text and ref. 5). Preparation of rabbit anti-mouse IgD is described in the text. Fluorochrome conjugates were prepared using DEAE-purified Ig fractions, and conjugates with fluorochrome: protein ratios of 1.5–2.5 were selected by chromatography on DEAE¹⁰.

all three categories of cells were represented in similar proportions.

Our findings in the mouse therefore mirror those in the human^{13,14}. In the mouse, there are fewer cells bearing both IgM and IgD, with correspondingly higher numbers of cells with exclusively IgM or IgD. These studies in the human, however, have been made with peripheral blood and so the comparison is not necessarily valid. The biological significance of these subpopulations of B cells should now be open to investigation. Since IgM precedes IgD in ontogeny, we favour the idea that in B-lymphocyte maturation there is differentiation from cells expressing only IgM to those expressing only IgD (ref. 5). An intervening cell type expresses both Ig classes. Thus the B lymphocytes of lymph nodes and Peyer's patches would in the main constitute a more mature population, possibly enriched with memory cells and precursors of cells destined to secrete IgG and IgA. The finding that IgA precursor cells of rabbit Peyer's patches are IgM and IgA negative, but certainly do have surface immunoglobulin¹⁵, suggests that these cells bear the rabbit equivalent of IgD.

The simultaneous expression of two Ig classes on the cell surface has implications for "switch" and V-C gene integration mechanisms. For example, the two constant region genes for μ and δ chains are known to share the same V region¹⁶, and if this reflects the simultaneous presence of two integrated genes (that is VH-C μ and VH-C δ), then a copy-choice mechanism for V-C gene integration¹⁷ is suggested.

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New evidence on the location of the saccharide-binding site of concanavalin A

THE biological activities^{1–4} of the mitogenic lectin concanavalin A (con A) depend on the binding of the protein to receptors on the cell surface, and this binding can be inhibited by specific saccharides related to D-mannose and D-glucose⁵. Knowledge of the location and nature of the saccharide-binding site of con A would therefore contribute greatly to understanding the molecular basis of its biological activities. Although the complete amino acid sequence^{6–8} and three-dimensional structure^{6,9–11} of con A are known, there has been conflicting evidence on the location of the carbohydrate-binding site. Crystallographic studies with saccharides labelled with heavy atoms indicated that it was in a deep cavity in the molecule, more than 20 Å from the Mn²⁺ and Ca²⁺ ions^{6,9,12} (Fig. 1). Subsequent magnetic resonance measurements^{13–15} as well as crystallographic considerations¹⁶ suggested that the site was 10–12 Å from the metals, and led to the hypothesis that the previously studied saccharides labelled with heavy atoms (for example, o-iodophenyl- β -D-glucopyranoside (β -IPG)) were bound by their hydrophobic aglycones, rather than by their saccharide moieties^{9,17}.

High concentrations of inhibitory saccharides crack or dissolve con A crystals of the kind used in the structure determination, precluding crystallographic observation of the

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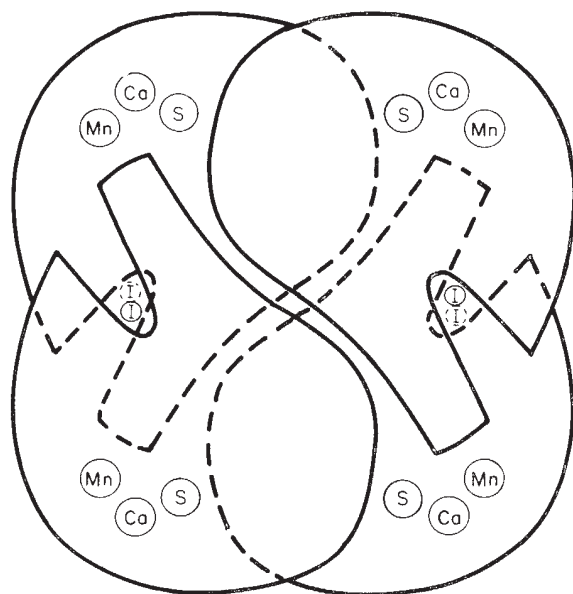


Fig. 1. Schematic representation of the con A tetramer. Each subunit is approximately $42 \times 40 \times 39$ Å, and the tetrameric complex possesses exact 222 symmetry. The manganese and calcium sites are indicated by Mn and Ca, respectively. The saccharide-binding site near the metals is indicated by S and the β -IPG-binding site in the cavity by I.

carbohydrate-binding site. We have therefore adopted several different approaches to locate the site. We describe here the results of three such experiments: examination of the structure of an insolubilised crystalline con A-saccharide complex, investigation of the structure of demetallised inactive con A, and preparation of a new crystal form of con A containing bound saccharides.

Although specifically bound sugars disrupt con A crystals of the type used in the original structure determination, we have carried out diffraction studies of carbohydrate binding using these crystals. Con A crystals, prepared as described before⁹, were treated with 1% glutaraldehyde for 1 h at room temperature. Addition of specific saccharides to these cross-linked crystals caused considerable changes in the diffraction pattern and a reduction of the limiting resolution from 2 Å to 3.5 Å (Fig. 2). These changes were reversed by soaking the crystals in saccharide-free buffer, and non-binding carbohydrates, such as methyl- α -D-galactopyranoside, did not produce such changes, suggesting that the alteration of the diffraction is caused by non-covalently bound specific saccharide.

Table 1 Comparison of locations of heavy-metal substituents in con A and its complex with 2-deoxy-2-iodo-methyl- α -D-mannopyranoside

Heavy-metal site*	Coordinates in native con A			Coordinates in the complex		
	x	y	z	x	y	z
Pb1	-13.67	-11.43	18.10	-14.19	-11.79	17.81
Pb2	-8.90	-22.59	11.16	-10.20	-22.10	11.37
Pt1	5.30	-0.35	3.66	5.57	1.04	4.42
Sm1	-13.58	-11.43	18.16	-13.36	-11.70	18.20
Sm3	-8.90	-23.29	11.67	-9.18	-23.05	12.41
II	0.45	-12.47	12.42	0.74	-12.91	13.32

*Coordinates are in Å referred to an origin at the 222 point of each protein system. Overall figure of merit for the 2-iodo- α -methylmannoside-con A phasing calculation is 0.714 for 1,894 reflections. Heavy-metal sites are named as in ref. 9, except II refers to an iodophenol derivative of the saccharide complex.

To avoid potential nonspecific binding to a hydrophobic aglycone, 2-deoxy-2-iodo-methyl- α -D-mannopyranoside¹⁸, an inhibitor labelled with heavy atoms, was used to form a con A-saccharide complex. Attempts to solve the structure of this complex with phases from native con A produced electron density maps that we could not interpret. Accordingly, new phases were calculated by multiple isomorphous replacement methods, and refined by electron density modification¹⁹. The molecular packing is like that of native con A, as indicated by the close similarity of the heavy atom positions in the two proteins (Table 1). In addition, a rotation function²⁰ calculated between native and saccharide-treated con A has its highest maximum at nearly zero net rotation.

Nearly all the polypeptide chain can be traced in the electron density map, and preliminary interpretation suggests that significant local differences in conformation are induced by carbohydrate binding. The positions of chain segments 95-105 and 195-207 relative to the rest of the molecule are altered, resulting in a disruption of the β -structure in this region. The entire metal-binding region seems to have moved 4-6 Å with respect to the rest of the molecule (Fig. 3). Local changes in this region cannot yet be described in detail, but there seem to be related changes in residues 33-45 and the top two chains of the back β -structure⁹. To locate the bound sugar molecule, a difference map between the complexes with the iodo sugar and its chloro analogue was calculated. The highest peak in this map was approximately 13 Å from the Mn²⁺ ion, at 0.74, 0.24, 0.42, which we interpret to be near to residues Gly 227 and Arg 228, in the metal-binding region of the molecule. Assuming that this peak represents the iodine of a bound saccharide molecule, the residues that may participate in con A-saccharide interactions are 14-16, 97-99, 168-169, 207-208, 224-228 and 235-237. These residues surround a shallow pocket near the top of the molecule (Figs 1 and 3)

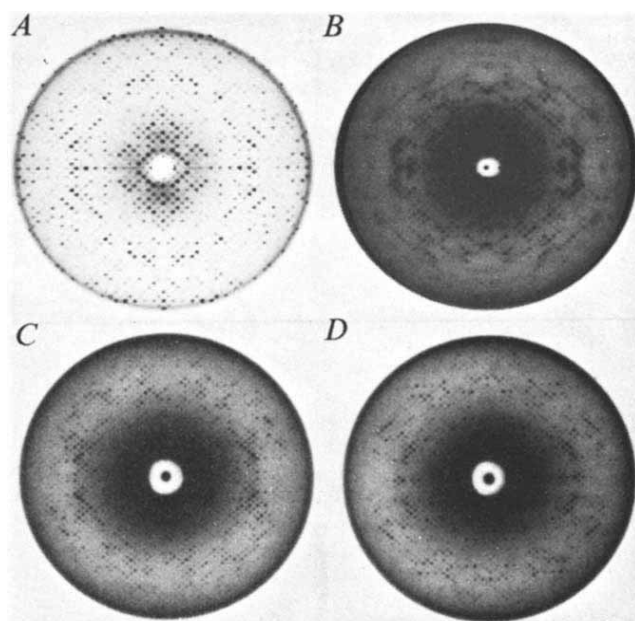


Fig. 2 A, $hk0$ zone of the native con A diffraction pattern. Reflections at the edge of the circle correspond to Bragg spacings of 2.8 Å. The space group is 1222 with $a = 89.91$, $b = 87.23$, $c = 63.07$ Å. B, $hk0$ zone of the cross-linked 2-iodo- α -methylmannose complex of con A. Space group 1222, with $a = 92.76$, $b = 86.66$, $c = 64.99$ Å. C, $hk0$ zone of trisaccharide con A crystallised from 20% saturated ammonium sulphate, pH 6.8. For comparison with D, a centred cell has been chosen, space group C1, $a = 119.04$, $b = 103.23$, $c = 132.09$ Å, $\alpha = 89^\circ 50'$, $\beta = 94^\circ 17'$, $\gamma = 85^\circ 03'$. D, $kh0$ zone of a con A crystal grown as in (C) and then treated with 50 mM α -methyl-D-glucopyranoside in 50% saturated ammonium sulphate. Space group C222₁, with $a = 118.09$, $b = 103.54$, $c = 251.8$ Å.

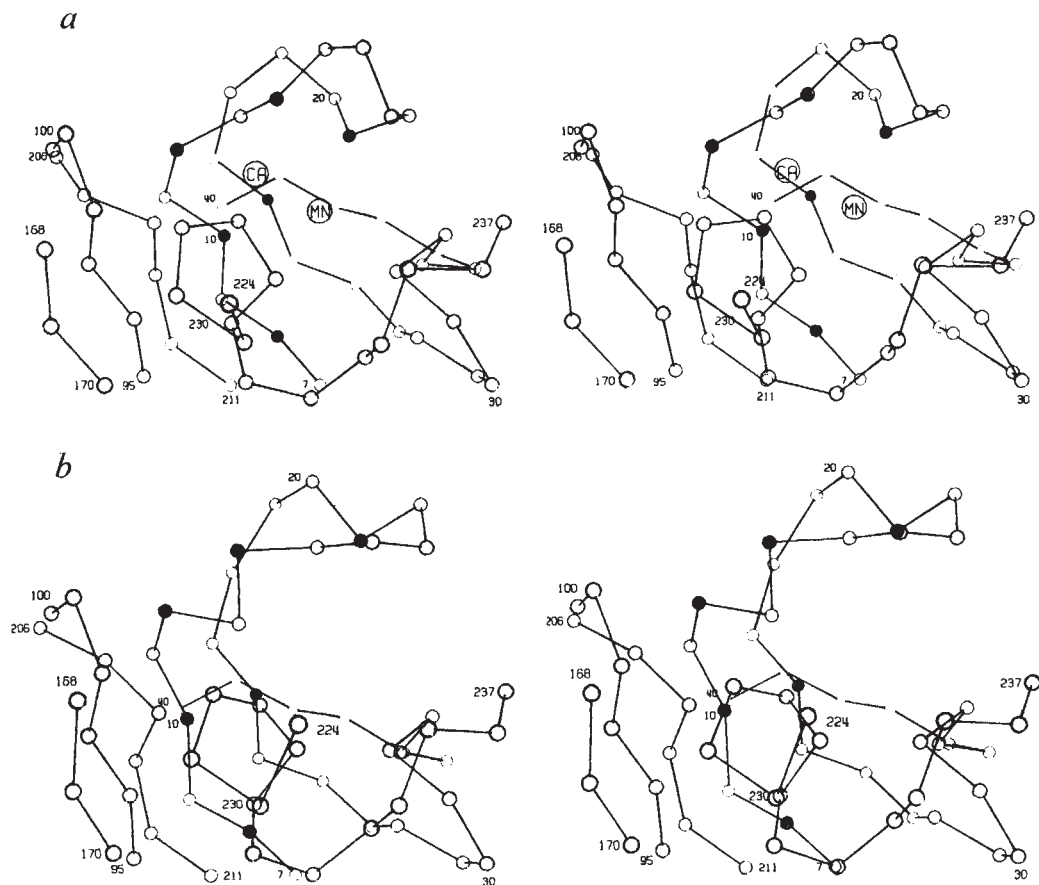


Fig. 3 *a*, Stereo drawing of the polypeptide backbone in the metal-binding region of native con A. ○, Alpha carbon atoms; ●, residues which serve as metal-binding ligands; CA and MN, calcium and manganese ions, respectively. *b*, The corresponding region of demetallised con A. The opening up of the polypeptide chain around the vacant metal-binding site is visible at the upper right.

and several of them participate in intermolecular interactions in the crystal. Disruption of these lattice interactions on saccharide binding may account for the difficulty of observing specifically bound saccharides in crystals of the type used in the original structure determination. The difference map shows no significantly large peak in the molecular cavity, suggesting that the binding site is elsewhere. This interpretation requires that the binding region in the cavity be a nonspecific site, active only in crystalline con A, inasmuch as in solution, hydrophobic sugars, such as β -IPG, are bound only at the specific site⁹.

The location of the site near the metal-binding region is supported by a study of the structure of demetallised con A. Removal of the bound Mn^{2+} and Ca^{2+} ions from native con A causes loss of saccharide-binding activity²¹⁻²³, suggesting that the saccharide-binding site is associated with a part of the con A structure that changes significantly with demetallisation. Demetallised con A was prepared^{23,24} and crystallised²⁵, and diffraction data to a resolution of 2.8 Å were collected as before⁹. The native and demetallised structures were aligned using molecular replacement methods. In separate experiments, structure factors were calculated for a native con A monomer or dimer in a triclinic cell with the demetallised unit cell dimensions. Complete rotational²⁰ and translational²⁶ searches indicated that the demetallised molecule was related to the analogous native con A dimer by a rotation of 7.5° about an axis near x , in agreement with earlier results²⁵, ($\alpha = 273.8^\circ$, $\beta = 7.5^\circ$, $\gamma = 87.7^\circ$ using Crowther's notation²⁰) and shifted slightly along z (translation = -0.0004, -0.0002, 0.0106 in the demetallised cell), the dimer moving as a single rigid body. (Details of the molecular replacement calculations will be published elsewhere.)

Structure factors calculated for this model, excluding residues in the metal binding region (residues 7-25), showed excellent agreement with the observed data for the demetallised molecule (conventional crystallographic $R = 42.6\%$, as compared with 40.4% for the final native con A structure). Difference maps

were calculated with coefficients

$$[(2|F_o| - |F_c|) \exp(ia_c)]$$

and

$$[(|F_o| - |F_c|) \exp(ia_c)]$$

where F_o are observed demetallised structure factors, F_c are model structure factors, and a_c are model phases. Examination of these maps allowed construction of a model of the metal-binding region and comparison of the two structures. The largest differences between the two structures were confined to the metal-binding region, the most significant being an opening of the tightly folded loop (residues 11-23) above the metal atoms, and a rearrangement of the nearby chains involving residues 99, 209, 228 and 237 (Fig. 3). The remainder of the molecule seems to be very similar in the two structures, with most of the changes being small (<1 Å) motions of the backbone or rotations of side chains. In particular, the folding in the region of the molecular cavity is almost identical. This localisation of significant structural differences between active con A and inactive demetallised con A strongly supports the hypothesis that the saccharide-binding site is in the vicinity of the metal-binding residues, approximately 13 Å from the Mn^{2+} ion, and not in the deep cavity (Fig. 1).

Definitive crystallographic proof of the location of the saccharide-binding site and its detailed characterisation must await the determination at high resolution of the structure of a con A-saccharide complex. To this end, we have prepared a new crystal form of con A that can bind specific sugars without loss of the high resolution diffraction pattern (Fig. 2). The striking similarity between diffraction from this crystal form and that from native con A suggests that solution of the structure by molecular replacement methods will be feasible. It is likely that this determination will confirm the interpretation suggested by the experiments described here that the saccharide-binding site is directly associated with the metal-binding

region of con A. This close association allows a simple explanation of the dependence of saccharide-binding activity on the presence of the metal ions, as the metals may directly stabilise the saccharide-binding residues in their active conformation.

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Concanavalin A blocks desensitisation of glutamate receptors on insect muscle fibres

CONCAVALIN A (con A), a tetrameric lectin which binds to specific carbohydrate residues¹, has been used in the purification of a glutamate-binding glycoprotein isolated from rat brain synaptosomes², and has been shown to alter the isotherm describing the binding of α -bungarotoxin to acetylcholine receptors on rat skeletal muscle³. Complementary electrophysiological studies on the effect of con A on intact receptor-bearing membranes are, as yet, lacking. We present here evidence that con A abolishes the desensitisation normally observed during repeated iontophoretic application of L-glutamate, the putative excitatory transmitter, to two populations of L-glutamate receptors on locust skeletal muscle fibres.

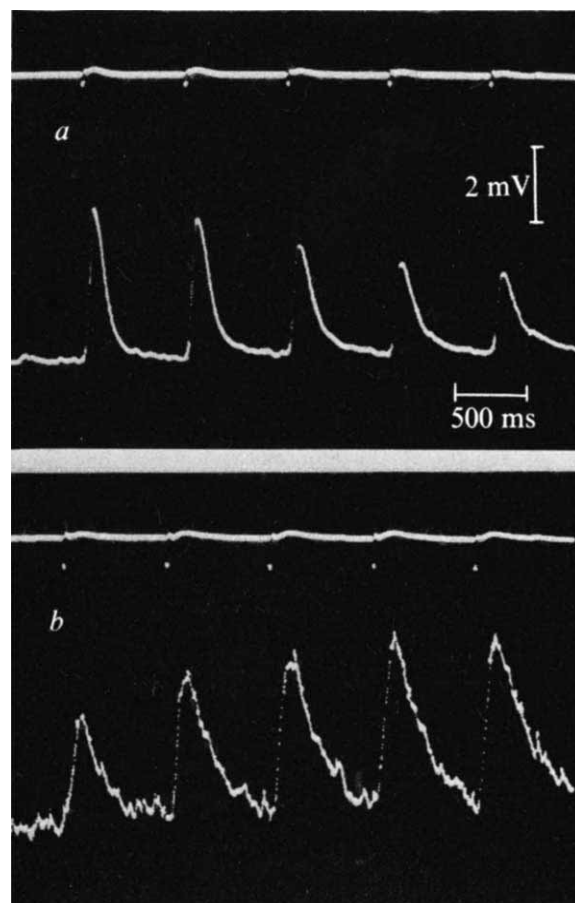
Iontophoresis of L-glutamate (pH 7.0, 1 M) through high resistance (120-300 M Ω micropipettes) is known to produce three types of response on innervated^{4,5}, as opposed to denervated⁶, locust extensor tibiae muscle fibres. When the tip of the glutamate electrode is within a few micrometres of a neuromuscular junction, a rapid transient depolarisation of rise time 10-50 ms and peak sensitivity ~ 100 mV nC⁻¹ is seen. This event is associated with the activation of excitatory junctional glutamate receptors^{4,5}.

These receptors exhibit desensitisation at glutamate pulse repetition rates ≥ 0.7 Hz (ref. 5 and Fig. 1a).

Iontophoresis of 1-5 nC of glutamate on to the extra-junctional membrane of locust muscle fibres evokes a biphasic response due to simultaneous activation of two populations of receptors designated as D-type receptors and H-type receptors, which are associated with ionophores for cations (D) and ionophores for Cl⁻ (H)^{7,8}. The D and H receptors occur evenly over the entire extrajunctional membrane, their sensitivity to L-glutamate iontophoresis being 0.2-0.5 mV nC⁻¹ (ref. 6), and both types are very readily desensitised by repeated pulses of iontophoretically-applied glutamate (Fig. 2a, b). Indeed, the diffusion of glutamate which occurs from drug-filled micropipettes of resistance < 100 M Ω is sufficient in itself to inactivate these receptors⁹.

Con A (Sigma, Grade IV), was dissolved in locust saline of composition 200 mM NaCl, 10 mM KCl, 2 mM CaCl₂, 4 mM NaH₂PO₄, 6 mM Na₂HPO₄, pH 6.8, and applied topically to the preparations at a concentration of 10⁻⁷ M. No change in membrane potential, input resistance or of frequency of spontaneous miniature potentials was observed. Within 0.5-1 h at 20-22 °C, the depolarising component of the biphasic extrajunctional response was no longer abolished by repeated pulses of glutamate. The H receptors, however, retained their normal rate of inactivation in these conditions (Fig. 3a-c). In sensitivity (0.2-0.5 mV nC⁻¹), rise time (40-60 ms) and homogeneous distribution over the extrajunctional membrane, the glutamate-

Fig. 1 a, Iontophoresis of 1-nC glutamate pulses at 720 ms intervals on to an excitatory junction of a muscle bathed in 10⁻⁷ M con A for 5 h. Responses showed desensitisation at a rate typical of junctional receptors in control fibres. b, Fibre exposed to 10⁻⁶ M con A for 1 h. Responses now exhibited simultaneous potentiation and summation to 1-nC glutamate pulses delivered at the same frequency as in a. Note high frequency of spontaneous miniature end plate potentials.



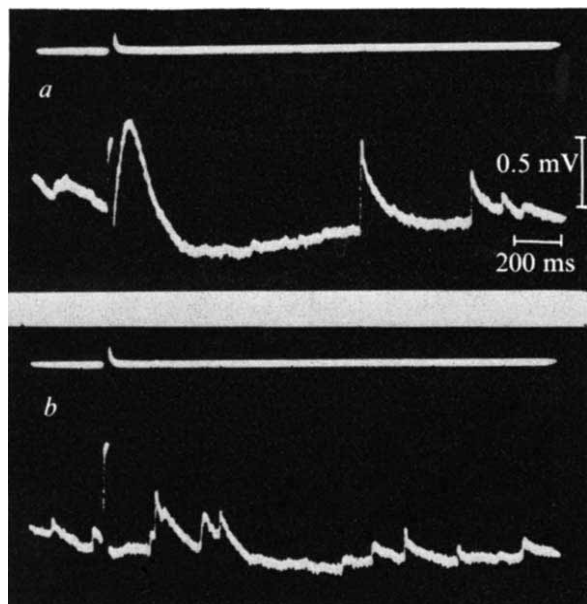


Fig. 2 *a*, Biphasic (D-H) response (lower trace) evoked by iontophoresis of a 5-nC pulse (upper trace) of L-glutamate on to the extrajunctional membrane of a locust muscle fibre. *b*, A second pulse, delivered 4 s later, produced only a small hyperpolarisation, indicating that both D- and H-receptor populations had undergone marked desensitisation. Miniature excitatory potentials are seen on the lower traces.

induced depolarisation in the presence of con A resembled closely the normal D response elicited from control preparations. Bathing the nerve-muscle preparation in saline in which Na^+ was substituted by choline reversibly abolished the D responses both in the presence and absence of con A.

The increased rate of application of an agonist is known to accelerate receptor desensitisation⁹. This phenomenon, also characteristic of both junctional and extrajunctional D receptors in control fibres of locust muscle, was not observed when glutamate was iontophored on to the extrajunctional membrane following exposure to 10^{-7} M con A. When the frequency of glutamate pulses was raised, potentiation of the D potentials occurred (Fig. 4*a*), while at still higher frequencies a summated form of response appeared (Fig. 4*b*), the amplitude of which was stable for pulse trains of < 20 s. This summated D response decayed rapidly to zero on switching off the iontophoretic current. The rise time of the summated D response in con A saline was reduced with increased rate of glutamate application (that is, increased pulse amplitude or frequency), while the amplitude and decay time were elevated by this procedure. Lateral movement of the glutamate electrode had no significant effect on the quantitative or qualitative aspects of this summated depolarisation, thus confirming its extrajunctional origin. The desensitisation of junctional glutamate receptors was not blocked by 10^{-7} M con A. On increasing the concentration of con A to 10^{-6} M, however, iontophoresis of glutamate on to excitatory synaptic sites evoked responses qualitatively similar to those seen on the extrajunctional membrane but of higher sensitivity and circumscribed origin (Fig. 1*b*). Since this concentration of con A also produced a significant rise in miniature potential frequency (see Fig. 1*b*), it is probable that the threshold concentration differential between D and junctional receptors reflects the presence of protective mechanisms at synaptic sites rather than a difference in the susceptibility of the two receptor groups to con A. The effects of con A on both junctional and D-receptor desensitisation could not be reversed by prolonged washing in con A-free saline.

Con A blocks the aggregation of β -lymphocyte immunoglobulin molecules which normally follows on their exposure to specific divalent antibodies¹⁰⁻¹². This inhibition is believed to be mediated by a submembranous microtubular array which forms as a consequence of the binding of con A to its own glycoprotein receptors¹³. We find it difficult to apply a mechanism of this nature to our observations in view of the normal desensitisation rate of the H receptors in the presence of con A. The H receptor is thought to possess structural features absent from the junctional and D-receptor populations. These presumably account for its activation by DL-isibotenic acid^{7,8}, a conformationally restricted glutamate analogue, and possibly its association with a Cl^- ionophore¹¹ whose conductance is blocked by picrotoxin. Con A may act by binding directly to junctional and D-receptor molecules, thereby preventing the conformational changes thought necessary for desensitisation to occur¹⁵⁻¹⁷, but leaving the initial molecular processes, associated with receptor activation and ionophore opening, unaffected. The structural features peculiar

Fig. 3 Iontophoresis of 5-nC pulses of L-glutamate on to the extrajunctional membrane of a fibre bathed in saline containing 10^{-7} M con A for 40 min. *a*, The first pulse elicited a biphasic response; the second and third pulses (*b*, *c*) delivered at 4-s intervals, showed progressive reduction in the hyperpolarising component due to desensitisation of the H receptors. There was no evidence for desensitisation for the D receptors in these conditions.

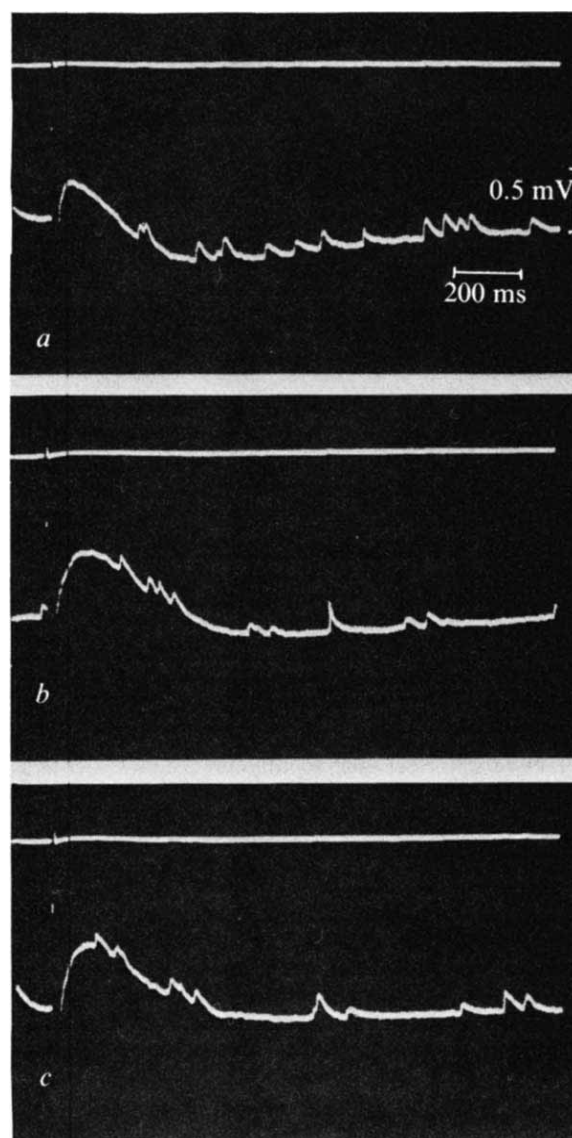
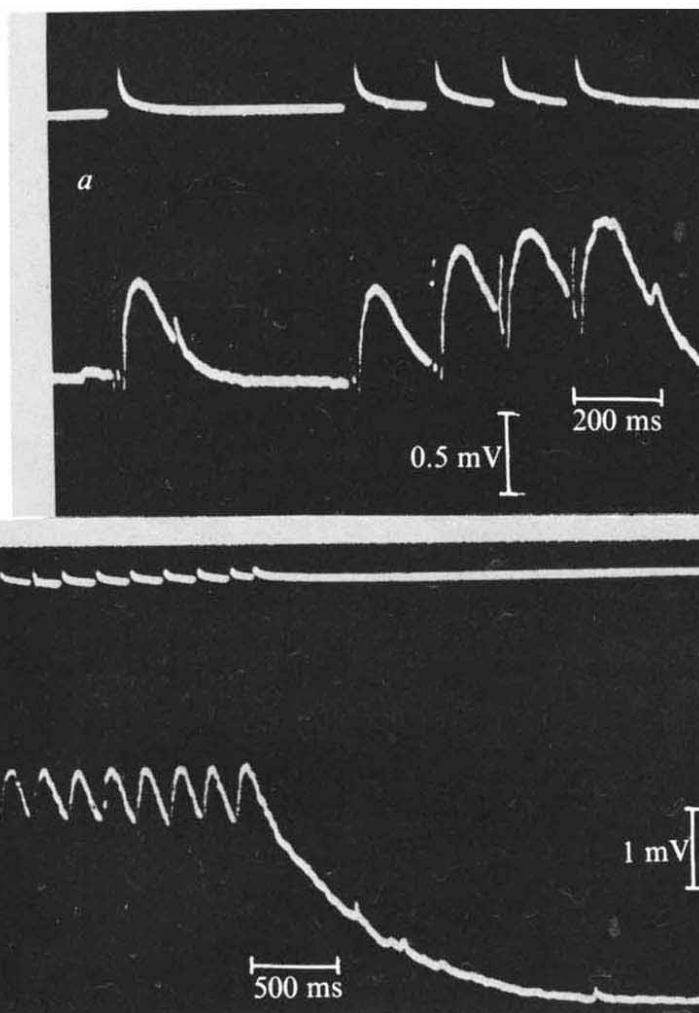


Fig. 4 Effect of glutamate iontophoresis, at various pulse intervals, on the response of the extrajunctional membrane after treatment with 10^{-7} M con A. On this fibre, there was no H-response present because of the equivalence of E_{Cl} and E_M . *a*, Two 8-nC pulses, delivered 650 ms apart, resulted in D responses of almost equal amplitude. After a further delay of 220 ms, a third pulse elicited a D response of potentiated amplitude. The fourth and fifth pulses, each occurring after a delay of 200 ms, produced less marked response potentiation. Summation is seen following the third and succeeding glutamate pulses. *b*, A prolonged train of 3-nC pulses, delivered at intervals of 240 ms, evoked a train of extrajunctional D responses. These depolarisations initially showed simultaneous potentiation and summation, leading to the formation of a stable, summated depolarisation which decayed rapidly on cessation of glutamate ejection.



to the H receptor may prevent the con A binding step.

It is likely that there are several glycoproteins on the surface of locust muscle membranes to which con A might bind. Perhaps the glutamate receptors on this membrane, like the putative glutamate receptors isolated from rat brain², are glycoproteins although this has not yet been established¹⁸. It is of interest that the binding of con A to rat brain glycoprotein cannot be reversed on simple washing, a feature shared with the desensitisation characteristics of locust muscle glutamate receptors that we observed. If the receptors on locust muscle are not glycoproteins, then the restriction of the conformational change which possibly leads to desensitisation of these receptors could be the result of con A binding to membrane glycoproteins closely associated with the receptors. Whatever the explanation for the action of con A on insect muscle, this substance will probably prove of great value in the further biochemical characterisation of glutamate receptors from both vertebrate and invertebrate sources. The drug may also provide a means of dissecting the molecular mechanisms underlying receptor desensitisation.

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Lithium inhibition of adrenaline-stimulated adenylate cyclase in humans

LITHIUM is an effective therapeutic agent in the treatment and prevention of the manic phase of manic-depression¹, and although known to influence many physiological systems, no specific mechanism has been identified for its efficacy in this illness². One postulated primary site of Li action is as an inhibitor of hormone-stimulated adenylate cyclase³. Li inhibits the ADH-sensitive adenylate cyclase in kidney^{4,5}, the TSH-sensitive adenylate cyclase in thyroid^{6,7}, the prostaglandin-sensitive adenylate cyclase in human platelets^{8,9}, and the noradrenaline-sensitive adenylate cyclase in rat brain^{10,11}.

It is therefore possible that therapeutic concentrations of Li in man inhibit adenylate cyclase activity. Ball *et al.*¹² have shown that administration of β -adrenergic agonists in

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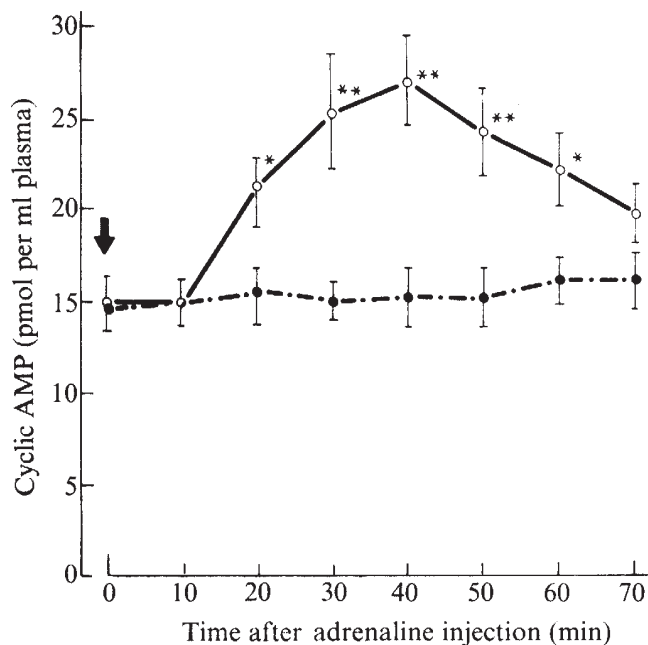


Fig. 1 The effect of Li on plasma cyclic AMP response to adrenaline administration. Subjects received 0.5 mg adrenaline subcutaneously at time 0 and blood was drawn every 10 min using an indwelling catheter in the antecubital vein. (Subcutaneous adrenaline injection was chosen rather than intravenous injection for greater clinical safety, in spite of the possibility of increased variability.) Samples were collected in plastic test tubes containing heparin and theophylline. Cyclic AMP was determined by the protein binding method of Brown *et al.*¹⁹ as modified for plasma by Lattner and Prudhoe²⁰. Bars indicate s.e.m. All subjects were physically healthy consenting adults. The lithium subjects (6 males, 3 females, ●) had a mean age of 38 ± 3.6 . Mean plasma lithium level²¹ was 0.71 mEq l^{-1} with a range $0.32\text{--}1.15 \text{ mEq l}^{-1}$. The no-lithium control subjects (7 males, 1 female, ○) had a mean age of 34 ± 3.6 . The lithium subjects included four manic-depressive patients and one unipolar depressive patient, all in euthymic states; a hypomanic manic-depressive patient; an euthymic schizo-affective patient; and two normal individuals. The no-lithium subjects included one euthymic manic-depressive patient; one depressed unipolar patient; one agitated schizo-affective patient; one hypomanic manic-depressive patient and five normal individuals. Comparison (not shown) of the response of normal individuals without lithium to the response of patients without lithium does not suggest any effect of diagnosis or clinical state on the plasma cyclic AMP response to adrenaline. * $P < 0.05$; ** $P < 0.01$.

healthy volunteers leads to a rise in plasma cyclic AMP. Plasma cyclic AMP is derived from intracellular cyclic AMP of several tissues including liver, muscle and adipose tissue, but the rise in plasma cyclic AMP after β -adrenergic stimulation seems to result largely from stimulation of β receptors other than those in the liver or adipose tissue¹³. We have measured the rise in plasma cyclic AMP induced by adrenaline in subjects on and off Li therapy, and have found that therapeutic doses of the drug block this effect.

The effect of adrenaline on plasma cyclic AMP levels for a group of Li-treated and control subjects is shown in Fig. 1. In the control subjects there was a mean rise in plasma cyclic AMP levels which commenced at 10 min after adrenaline injection, peaked at 40 min, and by 70 min had almost returned to base levels. Li-treated subjects showed no mean increase in plasma cyclic AMP levels after adrenaline administration. Examination of individual responses (not shown) reveals that each of the no-lithium subjects showed a rise in plasma cyclic AMP levels after adrenaline injection, the magnitude of the maximum response ranging from 38 to 221%. In contrast, five of nine Li-treated subjects showed no rise in plasma cyclic AMP levels and the remaining four only a small increase (29–

39%). It should be noted that there seems to be no effect of Li on basal adenylate cyclase activity, but only on the activation of this enzyme by the hormone. Similar results have been reported for the ADH-sensitive adenylate cyclase in the kidney⁴. Figure 2 shows that two normal individuals (R.E. and H.B.) who respond to adrenaline with a rise in plasma cyclic AMP levels ceased to show a response when the test was repeated after 3 d of Li treatment. This suggests that the effect of Li on adrenaline-stimulated adenylate cyclase is a pharmacological effect of the drug and is not dependent on pre-existing pathology.

In addition to a direct effect of Li on adenylate cyclase, it has been proposed that this drug may also act at a site more distal to the synthesis of cyclic AMP^{14,15}. Our results, however, suggest that a more distal action of Li need not be postulated to explain the effect of Li on the adrenergic system. Li's marked effect on β -adrenergic adenylate cyclase contrasts with the paucity of reports of Li effects on the peripheral cardiovascular system¹⁶. Intracellular levels of cyclic AMP after receptor stimulation are probably many times greater than those occurring in extracellular fluid¹². It is possible that intracellular cyclic AMP responses, even after Li administration, are adequate to maintain homeostatic integrity. Fann *et al.*¹⁷ have shown, however, that Li does decrease the pressor response to infused noradrenaline in man by 22%.

The catecholamine hypothesis of affective disorders postulates that mania is characterised by a functional excess of noradrenaline at a site in the central nervous system¹⁸. We have shown that in man, therapeutic doses of Li inhibit the peripheral β -adrenergic-stimulated adenylate cyclase. The brain adenylate cyclase stimulated by noradrenaline and the peripheral β -adrenergic-stimulated adenylate cyclase may be related, since both synthesise cyclic AMP after

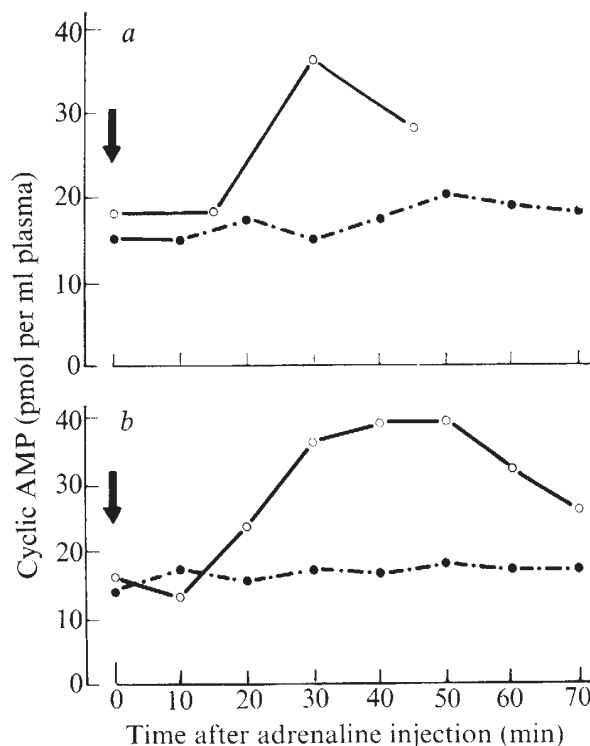


Fig. 2 The response of plasma cyclic AMP to adrenaline before (○) and after (●) Li in two normal subjects. After initially testing the response of plasma cyclic AMP to adrenaline in the absence of Li, both subjects were given Li for 3 d until a blood level of 0.60 mEq l^{-1} (R.E.) and 0.66 mEq l^{-1} (H.B.) was attained. The test was then repeated. The experimental procedure is the same as described in the legend to Fig. 1. a, Patient R.E.; b, Patient H.B.

hormonal stimulation. This is in contrast to stimulation of the peripheral α -adrenergic receptors, by either adrenaline or noradrenaline, which leads to a rise in cyclic GMP levels in plasma¹². If Li inhibits human brain adenylate cyclase stimulated by noradrenaline, its antimanic efficacy could be understood.

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Antagonism between channels for pattern and movement in human vision

MACKEY¹ rediscovered a striking experimentally produced visual hallucination. After inspection of stationary black-and-white stripes, a uniform test field is apparently filled with particles in chaotic streaming motion at right angles to the orientation of the stripes². A great deal has since been discovered about pattern-analysing channels in the human brain, and much of this information has been gained from the study of visual illusions and after-effects. The streaming hallucination, however, has never been properly explained³, and is now not much more than a curiosity in the textbooks. In this paper an explicit model of the streaming after-effect is proposed, and from it some new subjective after-effects are derived.

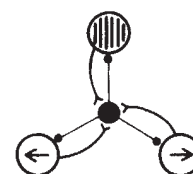
Physiological⁴ and psychophysical⁵ results imply that the visual system is organised into parallel subsystems of 'sustained' and 'transient' channels which have complementary roles in visual processing. Sustained channels code information about spatial form, pattern and contour, whereas transient channels primarily carry information about temporal change, flicker and movement. Sustained channels have higher spatial acuity, and selectivity⁶, whereas transient channels have higher temporal acuity, and in man are probably always direction selective⁶.

In the visual cortex, cells tuned for a particular contour orientation are grouped together in slab-shaped columns, along with cells whose 'preferred' direction of movement is

at right angles to that orientation⁷. Neighbouring columns have adjacent preferred orientations, producing a complete array of orientation columns⁸. We also know that mutual inhibition between neurones in a particular neighbourhood is a prime feature of cortical organisation⁹.

Suppose that, within a column, sustained (pattern) channels selectively inhibit movement channels and vice versa, as shown schematically in Fig. 1. It has frequently been suggested that to explain the motion after-effect ('waterfall illusion') opponent pairs of movement analysers are required¹⁰⁻¹², so that in one sense the triplet model of Fig. 1 may be seen as an outgrowth of that idea. If the output of one channel is reduced by adaptation, then the resting level of the other two channels must rise since they are released from inhibition. Therefore, after adaptation to stationary vertical stripes there is a temporary increase in the outputs of the horizontal movement channels and horizontal streaming movements are seen. After inspection of moving stripes one of the movement channels is also adapted; only the opposite movement channel is released from inhibition, and streaming is seen in that direction only².

Pattern channel



Movement channels

Fig. 1 Model of antagonism between cortical channels extracting components of pattern and movement in retinal image. There is mutual inhibition between a pattern channel selective for vertical contours and movement channels sensitive to left or right movements. The three channels illustrated can be expected to lie within a single cortical orientation column. Other orientations would be handled by similar triplets in neighbouring columns. A single inhibitory interneurone is shown (black disk) but other ways of producing the interaction are possible (for example, an interneurone for each pair of channels) without altering the basic idea. The model can explain the streaming after-effect^{1,2}, and predict some new phenomena (see text).

From the symmetry of the model two predictions can be made. Adapting to left and right movements without contours should release the corresponding pattern channel. Hallucinated stationary stripes at right angles to the movement should be seen as an after-effect. If adaptation was to only one direction of movement, then the hallucinated stripes should appear to drift in the opposite (unadapted) direction.

These predictions were tested on human observers by adapting to rotating dot patterns without coherent contours. Such a procedure should produce adaptation of movement channels with little corresponding pattern adaptation. Since the motion was circular, the predicted effect must be one of radial lines, at right angles to the adapting motion.

Two coarsely textured disks were seen superimposed by a half-silvered mirror close to the observer's eyes. Both disks rotated at 66 r.p.m. either in the same direction (clockwise) or in opposite directions. These conditions will be called single and double rotation respectively. After fixating the centre of this display for 1 min, the subject then gazed at the centre of a sheet of smooth white paper to observe any after-effects. His descriptions were recorded on tape and he also made drawings wherever possible. Each subject made two observations in each condition of rotation, and

these conditions were presented alternately. Half the subjects began with single rotation and half with double rotation.

After inspection of irregularly patterned disks rotating in the same or opposite directions most subjects spontaneously reported seeing complex patterns of radial lines or curves. There were also reports of fields of moving dots, or of no pattern at all. The taped reports were therefore analysed according to a fivefold classification: (1) stationary radial pattern, (2) moving radial, (3) stationary granular, (4) moving granular, (5) no pattern. After double rotation most reports were of stationary radial contours (50%) or of no pattern at all (Fig. 2). Two subjects reported patterns with some anticlockwise movement, but this was almost certainly a residual effect from the preceding trial of single rotation.

After single rotation half the reports were again of radial contours, but rotating anticlockwise, in the opposite

direction to the adapting motion. All but one of the remaining reports were of a field of dots in anticlockwise motion. In 80% of cases, the two observations in each pair fell into the same category of report; in the remaining cases, the first report was of no pattern or of moving dots, but the second report was of radial contours.

These results strongly support the proposed model of a mutual antagonism between three types of channel contained in each individual orientation column of the visual cortex. Adapting out one or two channels produces an 'hallucinated' after-effect based on the disinhibited or rebound response(s) of the remaining channel(s). Adapting to movement produces orthogonal contours (and vice versa, as MacKay's work¹ showed).

That a rotating field of dots (rather than contours) could be evoked suggests that sometimes only one of a disinhibited pair of channels may actually exceed the perceptual threshold. In this case it seems that the movement channel exceeded threshold but the pattern channel did not. As in the streaming after-effect² this seems to be characterised by the perception of moving granules. This interpretation is also consistent with the fact that some subjects continued to see movement for some time after the contours had faded.

Usually the after-effect would last only a few seconds, but during that time it could appear remarkably clear and vivid. Some of the subjects' drawings are reproduced in Fig. 2. Like subject 10, I see the lines as straight after double rotation but curved after single rotation. This difference was reported by several other subjects, but quite often curved lines were described even after double rotation. Following single rotation the apparent rotation of the hallucinated lines is about an order of magnitude faster than the conventional motion after-effect. In all cases the lines are confined to the adapted region of the visual field, and follow Emmert's law of apparent size when 'projected' on to a uniform field at different distances.

I have experimented less formally with dichoptic adapting conditions. Using a Dove prism, it was easy to adapt one eye to clockwise rotation while the other eye adapted to anticlockwise. Testing each eye separately I saw a pattern of radial curves rotating in a direction opposite to the adapting direction seen by that eye. That is, the adaptation seemed to be eye specific. Viewing the test field binocularly, however, I saw a stationary pattern of straight radial lines, just as obtained with binocular adaptation to double rotation. These observations were confirmed many times. Furthermore, as with the streaming after-effect, these contoured hallucinations did not transfer between the eyes, if one eye was adapted and the other was tested.

These observations with dichoptic adaptation can be understood if channels in the human brain have varying degrees of ocular dominance, as found for cells in cat and monkey cortex⁷. Testing the after-effect with one eye revealed the activity of monocularly dominated channels adapted to only one direction and thus adaptation seemed to be eye specific. If the tested eye had not been adapted, then no effect was seen, again implying eye-specific adaptation. Binocular testing did not give the sum or mixture of the two monocular percepts, but instead revealed the activity of binocular channels adapted to both directions. Thus the after-effect was stationary just as with ordinary binocular adaptation.

The subjective patterns and streaming movements generated by adaptation are therefore anything but a textbook curiosity. We can apparently use them to probe the columnar organisation and within-column interactions in the human visual cortex. There is considerable evidence for a more widespread inhibitory interaction between columns in the cortex, and this too shows up in the 'hallucinated' after-effects. Adapting to a sinusoidal grating gives rise to subjective patterns of other orientations and

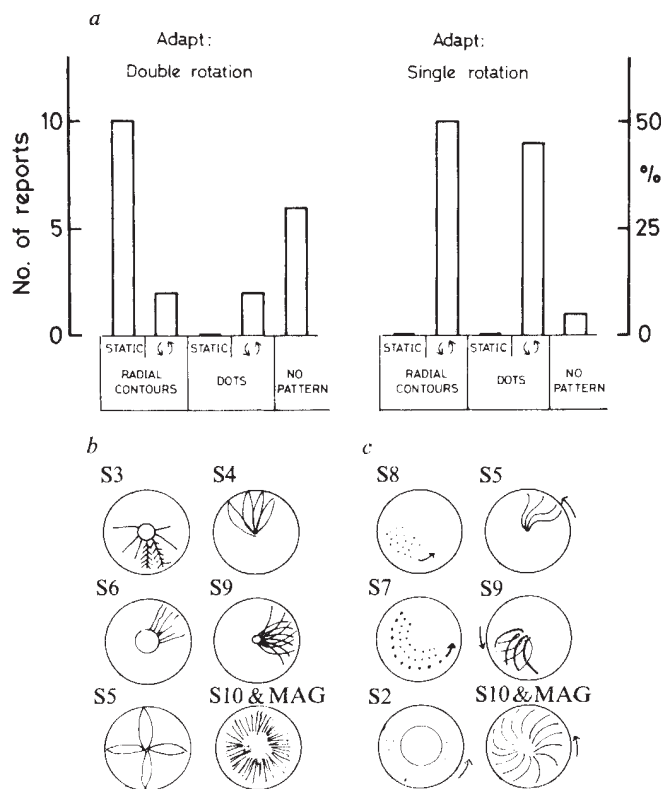


Fig. 2 *a*, Reports obtained from 10 naive observers after inspection of two superimposed dot patterns rotating clockwise (single rotation) or in opposite directions (double rotation) at 66 r.p.m. Disks subtended 12° at a viewing distance of 109 cm. Each disk bore a pattern of roughly circular dark spots on a white background. Each spot was about 0.5° across, drawn by hand in a quasi-random distribution, and separated by $0.5-1.5^\circ$. The contrast of each spot was about 0.4, relative to the combined background luminance (L_{max}) of 26 cd m^{-2} . (Contrast in this situation is defined as $(L_{max} - L_{min})/L_{max}$, where L_{min} is the luminance of the spot.) Patterns of smaller dots, or random visual noise were found to produce similar effects. *b* and *c*, Representative sample of subjects' sketches (redrawn). Where the lines fill only one quadrant of the drawing the observer invariably indicated that the whole region was filled with the same pattern. *b*, Adapting to double rotation produced stationary radial contours as an after-effect. *c*, Adapting to clockwise rotation produced radial contours (or a field of dots) rotating anti-clockwise. Appearance of curved lines (or cross hatching) as well as straight radial lines suggests that several near-radial orientations may be simultaneously disinhibited. The fact that the adapting speed increased with radial position on the disk might also have influenced the forms observed.

spatial frequencies¹³. These phenomena will be discussed in detail in a later paper.

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Effects of ethanol injections on morphine consumption in morphine-preferring rats

If there is a connection between the systems influencing morphine and ethanol consumption as suggested by the results of refs 1–4, then injections of ethanol should affect the intake of morphine. We have tried to determine if alcohol would cause a depression in morphine consumption in morphine-preferring rats similar to the results reported with ethanol consumption and morphine injections³. The results show no such connection to exist.

The subjects were 17 male Hooded rats (Canadian Breeding Farms) weighing between 250–325 g at the start of the experiment. All rats were housed individually in stainless steel cages. Purina Lab Chow was available *ad libitum*.

In the first phase of the experiment, a preference for morphine solutions over tap water was established. Since oral intake of morphine is limited by the reluctance of animals to ingest opiates presumably because of their aversive taste^{5,6}, we developed two procedures where non-nutritive saccharin was used to mask the taste of morphine. Eight rats were provided with a free choice between water and a morphine solution (0.1 g ml⁻¹) adulterated with 0.5 mg ml⁻¹ sodium saccharin, both of which were continuously available. The criterion used for morphine preference was arbitrarily set at persistent consumption of 60% or more of the total fluid intake as morphine. If this level was achieved, the morphine concentration was increased to 0.2 g ml⁻¹, whereas the saccharin concentration remained constant. If the rat continued to drink the morphine solution, its concentration was increased in steps of 0.1 g ml⁻¹ until a cutoff concentration of 0.5 g ml⁻¹ was attained. The 5 rats which developed a morphine preference comprised group 1. An additional 10 rats were provided with a free choice between tap water and a morphine solution (0.5 g ml⁻¹) adulterated with 0.1 g ml⁻¹ of sodium saccharin. If the rats failed to drink 60% or more of their total fluid intake from the morphine/saccharin tube, the saccharin concentration was increased in steps of 0.1 g ml⁻¹ until the criterion for preference was attained, or until the arbitrary cutoff concentration of 0.65 g ml⁻¹ of saccharin was reached. The morphine concentration was kept constant throughout. Four animals developed a preference for the morphine/saccharin solution and subsequently made up group 2. Group 3 (*N*=8), a control group, was given a free choice between tap water and a 0.5 g ml⁻¹ solution of sodium saccharin.

Seven days after group 1 and group 2 developed a preference for the saccharin-adulterated morphine at their

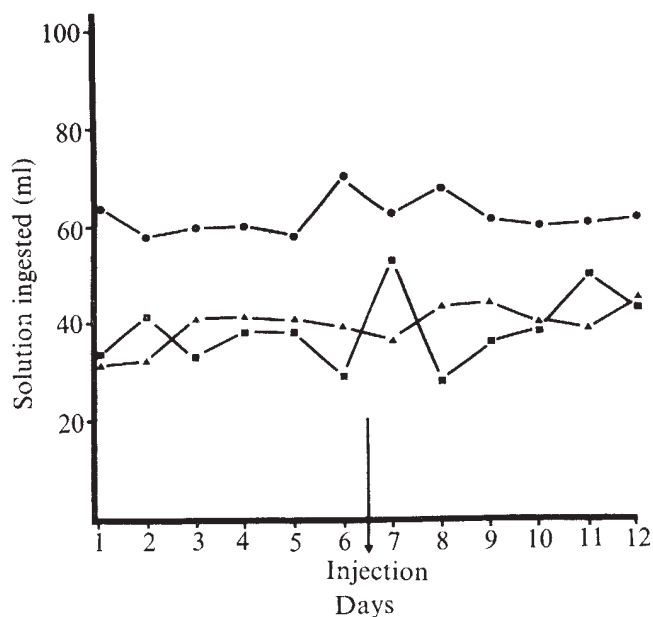


Fig. 1 Effect of ethanol injections on morphine intake over days. ●, Group 1; ▲, group 2; ■, group 3. The arrow represents injection of ethanol.

respective cutoff concentrations, the rats were injected intraperitoneally with 4 ml kg⁻¹ of 20% ethanol. Group 3 was given intraperitoneal injections of 20% ethanol (4 ml kg⁻¹) after having consumed 60% or more of their fluid intake from the saccharin tube for seven days. Fluid intake was recorded for six days following the injection for all groups. Food intake for the groups was recorded seven days preceding injection of ethanol and for a 6-d postinjection period. Fluid and food intake were monitored daily the same time throughout the experiment.

Seven days preceding injection of ethanol group 1 drank a mean of 60.38 ml of saccharin-adulterated morphine. The mean consumption of the saccharin-adulterated morphine for 6 d after the injection was 62.76 ml. A Wilcoxon-signed ranks pairs test indicated that there was no significant difference in morphine/saccharin intake between the preinjection and postinjection periods ($T=3$, $P>0.05$). Similarly, food and water were not affected by the injection ($T=5.6$, $P>0.05$; $T=5$, $P>0.05$, respectively). In the 7-d preinjection period, group 2 drank from the morphine/saccharin tube a mean of 33.95 ml. The mean saccharin-adulterated morphine consumption for the 6-d postinjection period was 41.0 ml. Again, there were no significant differences in morphine/saccharin consumption between pre- and postinjection periods ($T=3$, $P>0.05$), and food and water intake were not affected by the ethanol injection ($T=0.8$, $P>0.05$; $T=2.0$, $P>0.55$ respectively).

Control group 3 drank a mean of 37.25 ml from the saccharin tube in the 7-d preinjection period although their mean saccharin consumption in the 6-d period after the injection was 41.3 ml. Ethanol did not affect their saccharin consumption ($T=10$, $P>0.05$), food consumption ($T=5.6$, $P>0.05$) or water consumption ($T=17$, $P>0.05$) (see Fig. 1).

The results of this study suggest that ethanol does not influence the oral consumption of saccharin-adulterated morphine. The assumption that a common connection exists between the systems influencing the consumption of morphine and alcohol does not seem to be supported by these results. Perhaps if the rats were exposed to the morphine solutions for longer periods of time, the alcohol effect would become manifest. In the present study rats were exposed to morphine solutions for about 40 d; Sinclair's subjects were exposed to alcohol for 141 d. Length of

exposure to a drug could conceivably be an important factor to consider in studies of this nature, and may explain the dichotomy between these findings and those of Sinclair. Furthermore, it is possible that a such commonality does exist, but that it is not a simple symmetric relationship.

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Production of human chorionic gonadotropin in HeLa cell cultures

THE ectopic synthesis of placental proteins in human cancer is of special interest because of similarities between neoplastic and embryonic cells. Moreover, the inappropriate synthesis of biologically active proteins and polypeptide hormones which are normally produced only in specialised tissues (endocrine glands) or at a particular stage of development may provide information on the control of gene activity. Ectopic production of human placental chorionic gonadotropin (HCG) has been reported in some patients with neoplasms^{1,2}. A cell line (Cha Go) established from an HCG-producing pulmonary carcinoma was shown to continue synthesis of the gonadotropin in culture^{3,4}. We have now found that HeLa₆₅ cells^{5,7} produce the β subunit of HCG as measured by a specific radioimmunoassay and that sodium butyrate enhances production.

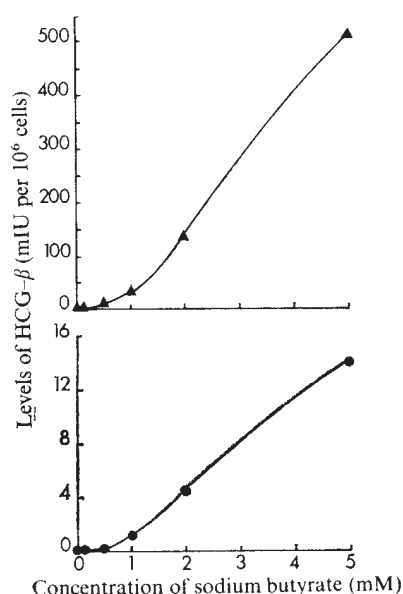


Fig. 1 Production of HCG- β by HeLa₆₅ cells grown for 120 h in the presence of different concentrations of sodium butyrate. ●, Intracellular HCG- β (mIU per 10^6 cells); ▲, HCG- β (mIU) secreted in 100 ml medium per 10^6 cells. HCG- β was determined by radioimmunoassay using polyethylene glycol as a precipitant for ¹²⁵I-labelled antigen-antibody complex. Radioactive complexes were measured as outlined in Table 1 by counting in a Nuclear-Chicago Gamma-Counter.

Table 1 Production of HCG- β by HeLa₆₅ cells

Addition to medium	Intracellular HCG- β (mIU per 10^6 cells)		HCG- β secreted in medium (mIU per 10^6 cell)	
	Mean	Range	Mean	Range
None	0.07	0.06–0.08	0.06	0.059–0.061
1 mM sodium butyrate	2.18	2.15–2.21	62.3	59.3–65.3
3 μ M prednisolone	0.14	0.136–0.144	0.10	0.099–0.101

Replicate flasks of HeLa₆₅ cells were grown for 120 h in Waymouth's medium containing 10% foetal calf serum and antibiotics (penicillin, 50 U, streptomycin 50 μ g and kanamycin 30 μ g ml⁻¹). The medium was decanted, freed from suspended cells by centrifugation and stored at -20 °C until assayed. The cells were removed from the glass surfaces with 0.02% EDTA and 0.05% trypsin (1:300 Grand Island Biological) in Puck's saline A. The cells were counted in a haemocytometer, washed by centrifugation in 0.15 M saline and frozen at -20 °C. Before assay the thawed cell pellet was suspended in 0.15 M saline and disrupted by sonification using Model W 185 Sonifier Cell Disrupter (Heat System—Ultrasonics). β subunits of human chorionic gonadotropin (HCG- β) were measured by a specific radioimmunoassay that exhibits less than 1% cross reaction with luteinising hormone (LH). Antiserum against the beta chain of HCG was used to ensure the specificity of the immunological reaction. Radioactive ¹²⁵I-HCG- β antigen antibody complexes¹⁶ were precipitated with either anti-gammaglobulin serum or polyethylene glycol (molecular weight 6,000). The radioactive precipitate was collected by centrifugation at 4,000g in a refrigerated (4 °C) centrifuge and counted in a Picker Nuclear Autowell II Gamma-Counter. The concentration of HCG- β in ultrasonicates of washed HeLa cells and in medium were calculated from a standard calibration curve. The standard curve was obtained by plotting %¹²⁵I-HCG- β bound to HCG- β antibody against known concentrations of unlabelled highly purified HCG- β . Standards were run concurrently with unknowns in each experiment. Purified HCG- β (molecular weight 22,000) and purified HCG- β labelled with ¹²⁵I and antibody against HCG- β were supplied by Serono Laboratories, and also by the Institute of Bio-endocrinology, Montreal. Values are the means and ranges of duplicate assays carried out on two replicate flasks.

HCG is a heteropolymer composed of two dissimilar subunits; an alpha subunit that is common to follicle-stimulating hormone, luteinising hormone, thyroid-stimulating hormone and HCG and a β subunit unique for each hormone that confers biological specificity⁵. Table 1 shows that HeLa₆₅ cells produce low levels of HCG- β and secrete it into medium. Growth of cells in medium containing sodium butyrate markedly increases HCG- β production both intracellularly and in medium (Table 1). Similar striking effects were observed in ten additional experiments. The amount of HCG- β produced by HeLa₆₅ cells depends on the concentration of sodium butyrate in the medium (Fig. 1). Sodium butyrate (0.1–10 mM), when added to the assay mixture, does not interfere with the radioimmune measurement of HCG- β . Another well characterised HeLa subline designated HeLa₇₁ (ref. 6) also produced HCG- β which was increased by growth in medium with sodium butyrate. On the other hand, the cortisol analogue, prednisolone, increases HCG- β only slightly. In four other experiments, prednisolone slightly increased the gonadotropin levels in one but was without significant effect in the other three. In baby hamster kidney BHK/C₁₃ cultures⁸ and adult human lung fibroblasts gonadotropin could not be detected nor could HCG- β be induced by growing these cells in medium with either sodium butyrate or prednisolone (data not shown).

It is interesting that the heteroploid HeLa cell line after many years in culture produces several proteins normally found only during embryonic development or synthesised by specialised cells. For example, these cells produce the placental form of alkaline phosphatase with electrophoretic characteristics similar to the isozymes of the heterozygous FS phenotype⁹. The addition of iron to culture media mediates the synthesis by HeLa cells of an apoferitin with properties different from the adult form of this protein¹⁰. HeLa cells are agglutinated by concanavalin A, a property shared by tumour and embryonic cells (our unpublished

results). The production of HCG- β by HeLa cells, as measured by a specific radioimmunoassay, provides additional evidence for the putative derepression of an element of the genome with the synthesis of a protein normally produced only by the specialised trophoblastic cells.

Cortisol^{11,12} and more recently sodium butyrate¹³⁻¹⁵ have been reported to modulate the shape and to alter the activities of several enzymes in HeLa cells. Short chain fatty acids such as butyrate increase the activity of HeLa alkaline phosphatase¹⁴ and sialyltransferase¹⁵. The accumulation of HCG- β in HeLa cell cultures is enhanced markedly by growth in medium containing sodium butyrate. Since this peptide hormone is not degraded in cell cultures⁴, it seems that the synthesis of HCG is increased by butyrate.

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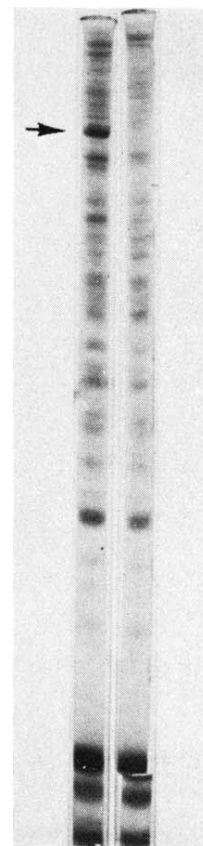
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Loss of a non-histone chromatin protein parallels *in vitro* differentiation of cartilage

SEVERAL lines of evidence have implicated the proteins associated with the nuclear DNA of eukaryotic cells in the control of differential gene expression¹⁻⁶. In contrast to the histones of eukaryotic chromatin which are relatively conserved in both primary structure and distribution across cell types within an organism, and from species to species⁴, the non-histone chromosomal proteins are very variable in size and distribution. This lends weight to the presumption that they are the specific regulators of transcription^{5,6}.

Although there have been studies of tissue-specific⁷⁻⁸, developmental stage-specific⁹⁻¹¹ and cell-cycle phase-specific¹²⁻¹⁴ variations in the complement of non-histone chromatin proteins, the causal association of specific alterations in chromosomal proteins with a particular transformation of one cell type into another can best be established with a homogeneous population of precursor cells able to differentiate in isolation into a homogeneous population of a recognisable cell type. In particular, differences in the chromosomal proteins of cell types which are adjacent in a developmental lineage would be more amenable to analysis than the larger number of differences likely to be encountered among cell types more widely diversified during development. Here we report a change in the complement of non-histone, chromosomal proteins in an *in vitro* system that closely approaches this situation.

Fig. 1 Gels prepared from chromatin proteins from nuclei of freshly explanted (left) and 3-d cultured (right) sub-apical ridge mesoderm from Hamilton-Hamburger stage 25 chick wing buds. Tissue was pooled from 370 White Leghorn embryos; nuclei were isolated from half the material immediately and half after 3 d in culture (see text). In each case intact pieces of tissue were suspended in a buffer consisting of 0.32 M sucrose, 0.001 M MgCl₂, 0.3% Triton N-101 and 0.001 M phosphate buffer, pH 6.8. Tissue was homogenised with two strokes of a Teflon pestle in a Potter-Elvehjem homogeniser. Nuclei were collected at 1,000g and resuspended in 3 ml of the same buffer, washed in 80 mM NaCl, 20 mM EDTA, pH 7.2, and then in 0.15 M NaCl, 0.05 M Tris pH 7.5. The resulting pellet was dissolved in 2% sodium dodecyl sulphate (SDS) in 0.01 M Tris and 10% glycerol and boiled, after which 1 mM phenylmethylsulphonyl fluoride^{21,22} was added. The mixture was then dialysed against 0.1% SDS, 0.01 M Tris, 0.3 M glycine, and run on Tris-glycine 10% polyacrylamide SDS gels using the gel and buffer systems of Laemmli²³. Approximately 150 μ g of protein was applied to each gel. Gels were stained with Coomassie brilliant blue. The protein characteristic of the prechondrocyte preparation is indicated by the arrow.



As described¹⁵, the mesenchyme subjacent to the apical ectodermal ridge¹⁶ of the stage 25 (5-d) chick wing bud consists of cells which are not chondrocytes according to several criteria. The dissociated cells cannot clone for cartilage in conditions which support cartilage colony formation by authentic chondrocytes¹⁷; sectioned subridge mesoderm does not stain metachromatically with toluidine blue, a test which is diagnostic for the sulphated mucopolysaccharides of cartilage matrix¹⁸; and most definitively, the collagen produced by the subridge mesoderm is of a level (1.5-2.5% of total newly synthesised protein when grown in ascorbate), and species (type I-like) uncharacteristic of cartilage (ref. 19 and R. Mayne, unpublished results). Nonetheless, after 3 d in organ culture, explanted subridge mesoderm attains the cartilage phenotype by the following criteria: dissociated cells clone for cartilage; sectioned explants stain metachromatically with toluidine blue, and the level (14-16% of newly synthesised protein) and species (type II-like) of collagen indicate that the tissue has virtually completely chondrified. At the histological level the transition proceeds uniformly during the 3 d, with moderate DNA synthesis at all stages (determined autoradiographically) and no overt necrosis. No myotubes are ever seen in these explants, suggesting that while the freshly explanted material is not yet committed to a chondrogenic programme, it has nevertheless passed the stage in the mesodermal lineage when it can 'choose' to be myogenic²⁰.

It is significant with regard to the possible mechanisms involved in this transition during development, that the individual α -helical chains comprising chick type I collagen (found in skin, tendon, bone and various other tissues) differ in their primary structure from the α -helical chains comprising chick type II collagen (found only in cartilage)¹⁹. The appearance of a new gene product in the subridge mesodermal cells or their descendants during

the 3 d in culture suggested reprogramming at the chromosome level and led us to look for corresponding changes in the nuclear proteins of these cells.

Tissue was isolated from the sub-apical ridge region of 370 stage 25 chick wing buds by transection of the limb tip 0.2–0.3 mm from the apex. No attempt was made surgically to remove the ectoderm which we estimate contributed less than 10% of total cells to the newly isolated material. Also, as sheets of ectoderm were lost during the early steps of nuclear isolation, the contribution of ectodermal nuclei to the final chromatin protein sample was probably a few per cent. Nuclei were isolated immediately from half the tissue, and from these chromatin was prepared and stored in a denaturing buffer containing phenylmethylsulphonyl fluoride, an inhibitor of proteolysis^{21,22}, at -20°C .

The other half of the tissue was cultured on 1.2% agar containing Ham's F-10 medium supplemented with 10% foetal calf serum and 1% bovine serum albumin. After 3 d in culture, nuclei were isolated from this tissue by the same procedure, chromatin was prepared, and the two preparations were run simultaneously on 25 cm 10% polyacrylamide gels containing 0.1% sodium dodecyl sulphate. The stained gels and the corresponding visible light spectrophotometer scans in Figs 1 and 2 were made from the preparation described, in which the freshly explanted and cultured material was taken from the same group of randomised embryonic limbs. Replicate analyses were done from separately prepared fresh and cultured tissue, yielding gels virtually identical to those pictured.

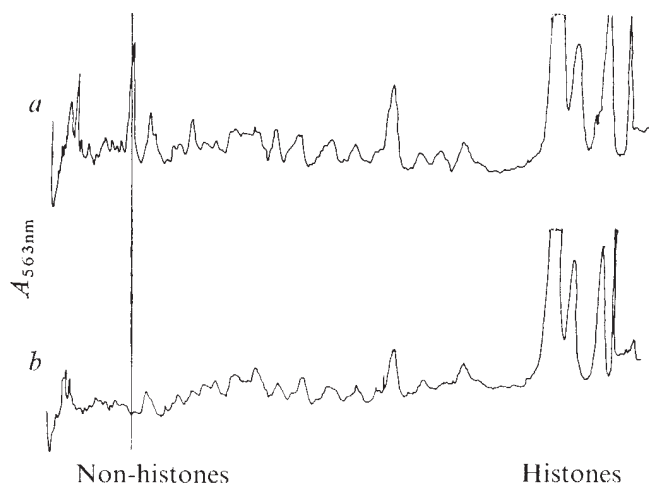


Fig. 2 Optical scans of the gels in Fig. 1 done at 563 nm on a Gilford spectrophotometer (a). Preparation from freshly explanted material. b. Preparation from cultured material. Tracing of prechondrocyte distinctive band is indicated by the line. The histone region may also contain some non-histone proteins.

In view of the phenotypic behaviour of the material analysed, we emphasise the following features of our electrophoretic data. First, we note the essential similarity between the two gels with respect to most of their stainable bands. While there are quantitative variations between some of the corresponding bands, this similarity appears greater than that usually found when different tissues or organs from a single individual or species are compared⁷⁻¹⁴. Second, the presence of a heavily staining band of low mobility (molecular weight between 125,000 and 150,000, estimated from markers in separate gels) in the freshly explanted, but not cultured material, is quite striking. This band is not present in similar gels of material prepared from cultured embryonic chick vertebral chondrocytes, fibroblasts or myogenic populations, though a low intensity band of similar mobility appears in chromatin preparations from primitive series chick erythrocytes (unpublished results of J.B., G.C.T.Y., and H. Holtzer). We do not exclude the possibility that the phenotypic differences between our freshly explanted and cultured material could be partly or entirely controlled by chromatin-associated molecules outside the limits of detection of our electrophoresis, staining, and scanning techniques. But we emphasise that our two preparations represented cell types which are adjacent in a developmental lineage and that the distinguishing protein (or class of proteins) was prominent against a relatively constant background, suggesting a role for this component in nuclear reprogramming. The all-or-none character of its disappearance (or loss of affinity for the stain) during the change from prechondrocyte to chondrocyte in culture recommends this system for *in vitro* studies of the mechanisms of cell differentiation.

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Expression of parental histone genes in the intergeneric hybrid *Triticale hexaploide*

Triticale hexaploide, the intergeneric hybrid resulting from the cross of diploid rye (*Secale cereale*) and tetraploid wheat (*Triticum durum*), offers a unique opportunity for the study of the expression of genes which are derived from two dissimilar organisms but exist together in the same cells. I have investigated the expression of histone genes in triticale because the histones are an extensively studied class of proteins with individual components which range from being highly conservative in an evolutionary sense to being probably species specific. For example, the sequence of histone F2a1 from peas and cows differs in only two of 102 amino acids¹, whereas different species of sea urchin have F1 histones of different electrophoretic mobilities². I

have found that in wheat, rye and triticale the evolutionarily conservative histones seem to be identical. There are, however, differences in the F1 histones of wheat and rye, and the gene for one of the wheat F1 histones is not expressed in the hybrid triticale.

Histones were extracted from dark-grown coleoptiles of triticale and from the rye and wheat parents as previously described³, and were analysed electrophoretically on short and long one-dimensional gels by the method of Panyim and Chalkley⁴ and on a new two-dimensional electrophoretic system.

Not surprisingly, considering the relatively close phylogenetic positions of wheat and rye, the highly evolutionarily conservative histones (F2a1 and F3) and the moderately conservative histones (F2b and F2a2) from all three organisms appeared identical on both one and two-dimensional gels (Figs 1 and 2). The only differences were in the F1 (lysine-rich) histones.

On short gels wheat F1 histones were resolved into four distinct bands, F1a, b, c and d. Long gels further resolved

wheat F1a into two electrophoretic bands, the slower of which I have called F1a'. This heterogeneity of wheat F1 histone is not due to phosphorylation since treatment of the isolated F1 fraction with alkaline phosphatase by the procedure of Sherod *et al.*⁷ did not affect the electrophoretic mobilities of the subfractions. Rye has only three F1 histones as resolved on both long and short gels. These have identical electrophoretic mobilities (as shown by electrophoresis of mixed samples of wheat and rye on long gels) to histones F1a, b and d (that is, rye histones are the same as wheat histones except that F1c and F1a' are lacking).

In the hybrid triticale the genes for histones F1a, b and d are expressed as is the gene for the wheat-specific F1a'. Even greatly overloaded gels, however, show no trace of the wheat-specific F1c histone.

The reason for the lack of expression of the wheat F1c gene is unknown, although it is not due to the loss of any whole chromosomes. Root tip squashes reveal the entire $2n$ complement of 28 chromosomes derived from wheat and 14 derived from rye, in the hybrid.

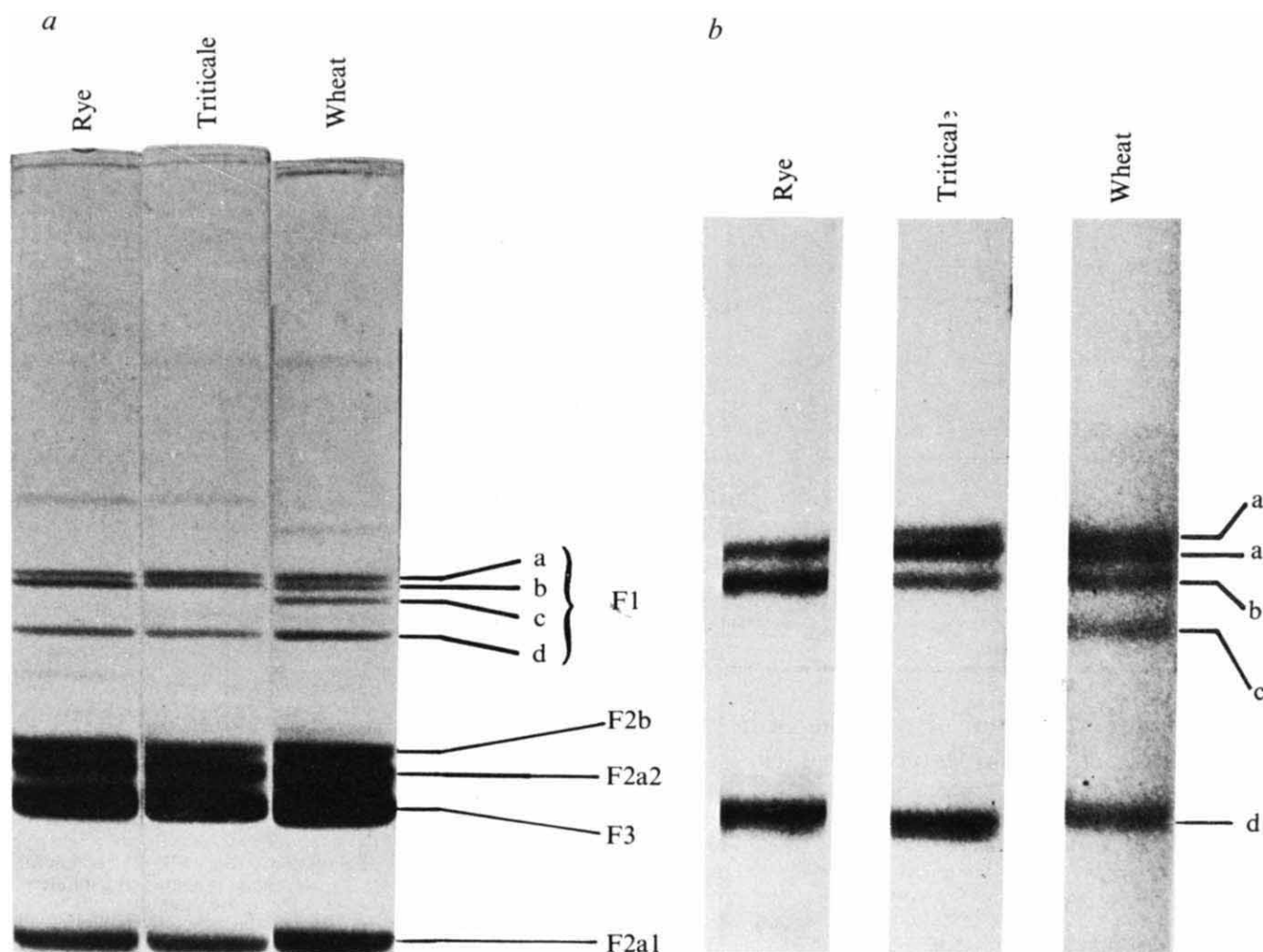


Fig. 1 *a*, Electrophoresis of wheat, rye and triticale whole histones on short gels by the method of Panyim and Chalkley⁴. The 10-cm gels were run for 4 h at room temperature, 2 mA per gel, then stained with amido black and destained by diffusion. All three species have histones F1a, b and d. The wheat-specific F1c is not expressed in the hybrid. The remaining histone fractions appear to be identical in all three plants. They have been named according to the nomenclature of Johns⁵ and identified by their fractionation properties, susceptibility to ferric chloride destaining and comparative mobility in one- and two-dimensional electrophoresis (manuscript in preparation). In this electrophoretic system the plant F2b and F2a2 histones have lower electrophoretic mobilities than histone F3 whereas histones F2b and F2a2 of mammals have greater electrophoretic mobility than histone F3 (ref. 3). *b*, Electrophoresis of wheat, rye and triticale F1 histones on long gels. The gels are of the same composition as the short gels in *a*, but are 20 cm long and were run at 0.75 mA per gel for 50 h at 2 °C. Other histone fractions have run off the gel, and only the portion of the gel containing the F1 histones is shown. Rye histones appear the same as in short gels but are separated further. F1a has been resolved into two bands in wheat and triticale, the slower of which has been labelled F1a'. The wheat-specific F1c is not expressed in the hybrid.

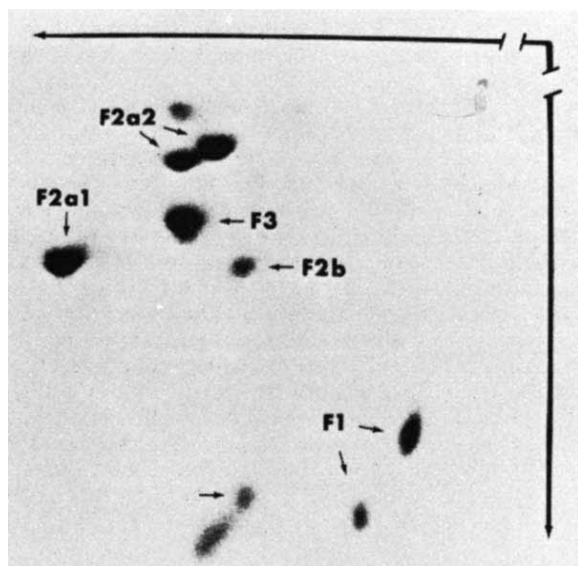


Fig. 2 Two-dimensional gel of tritacale whole histone. Separation in the first dimension (right to left) was on acetic acid-urea gels according to Panyim and Chalkley⁴. The second dimension (top to bottom) was of the same composition but included 1% Triton X-100. The patterns for rye and wheat are identical except for the F1 histones. The rapidly moving, arrowed but unlabelled spots are probably histones which have not bound the Triton⁶.

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Transbilayer movement of cholesterol in dipalmitoyllecithin-cholesterol vesicles

THE movement of cholesterol across biological membranes is an important process for many cellular functions, but the mechanisms by which it occurs are not known. The movement of lipid molecules between the two halves of a bilayer, a process which has come to be known as flip flop, was first demonstrated to occur in films of stearate¹. Subsequently, several workers have reported flip-flop times for phospholipids in model systems, the values obtained varying between several hours and many days depending on the system studied and the techniques used²⁻⁵.

Lipid flip flop provides a possible mechanism for the translocation of cholesterol across membranes. To our knowledge only one study has been carried out to estimate the rate at which such a process could occur. Smith and Green⁶ reported a half time of 70 min at 30 °C for the flip flop of the fluorescent cholesterol analogue, sterophenol, in liposomes. We have carried out experiments to measure the flip flop of cholesterol itself in sonicated dipalmitoyllecithin-cholesterol vesicles, a

well known system⁷. On the basis of two kinds of experiments using different strategies, we conclude that cholesterol flip flop in this system is a very slow process if it occurs at all.

Lipid vesicles were made by sonication of dipalmitoyllecithin (DPL) (Sigma) and cholesterol in the mol ratio of 1:0.9 at 4 °C for 1 h under nitrogen, followed by centrifugation at 20,000g for 45 min at 4 °C. The purity of the DPL was checked by thin-layer chromatography before and after sonication, and there was no evidence of impurities or chemical degradation. Red-cell ghosts were prepared using the technique of Dodge⁸. The ghosts were used in excess as an acceptor membrane and no attempt was made to measure their leakiness. Red cells were labelled with ¹⁴C-cholesterol by incubating them in plasma containing ¹⁴C-cholesterol⁹. Lipids were extracted from red cells⁸ and from ghosts¹⁰, and cholesterol was determined by digitonin precipitation¹¹ and assayed by the method of Parekh and Jung¹².

In the first set of experiments DPL-cholesterol vesicles containing ³H-cholesterol and ¹⁴C-DPL were incubated at a cholesterol concentration of 10⁻⁵ M with red-cell ghosts at a cholesterol concentration of 4.2 × 10⁻⁴ M. This represented a 42-fold excess of ghost membranes, so that back exchange of the tritiated cholesterol would be insignificant. The ghosts and vesicles were incubated at pH 7.4 in a phosphate buffer (1.2 mM MgCl₂, 2.4 mM CaCl₂, 4.2 mM NaH₂PO₄, 1.7 mM Na₂HPO₄, 5.0 mM KCl, 13.5 mM Na₂CO₃, 117.8 mM NaCl, 10.0 mM glucose) at 37 °C. At various times aliquots were taken, the ghosts separated from the vesicles by centrifugation, and both the supernatant and pellet assayed for ³H and ¹⁴C. To prevent sticking of the vesicles to the ghosts, 10⁻⁶ M bovine serum albumin was added to the incubation medium. Figure 1a indicates that the exchange phenomenon saturates after 73% of the ³H-cholesterol has been removed from the vesicles, in ~ 24 h. No additional cholesterol could be removed from the

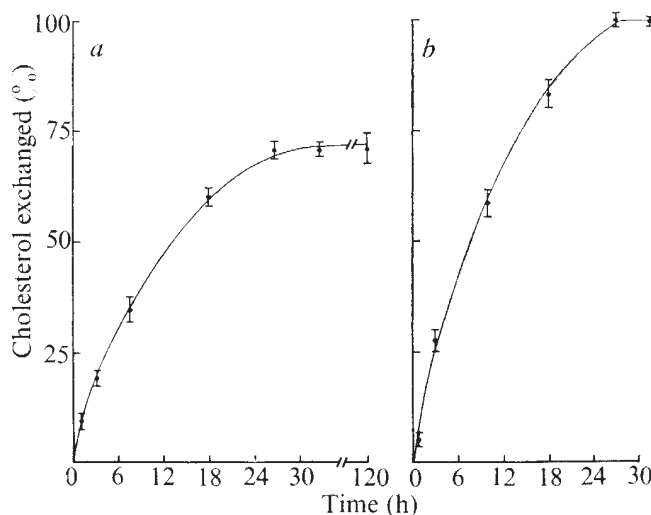


Fig. 1 *a*, Exchange of ³H-cholesterol from DPL-³H-cholesterol vesicles into erythrocyte ghosts where the labelled cholesterol was introduced into the original sonicated mixture. % cholesterol exchange refers to the amount of vesicle cholesterol transferred to the acceptor membrane. Correction (never exceeding 7%) was made for vesicles sticking to ghosts at each time point. *b*, Exchange of ³H-cholesterol from DPL-cholesterol vesicles into erythrocyte ghosts where the labelled cholesterol was introduced into the vesicles by a previous incubation with intact red cells containing ³H-cholesterol. The vesicles were stored at 37 °C for 48 h before being added to the ghosts preparation. The incubation medium contained streptomycin sulphate as an antibacterial agent and 10⁻⁶ M bovine serum albumin to reduce sticking of the vesicles to the ghosts. ¹⁴C-DPL was used as a control to monitor the degree of sticking and the concentration of phospholipid in the supernatant containing vesicles. The disappearance of labelled cholesterol from the vesicles corresponds with the appearance of label in the ghost fraction.

Table 1 Ratio of ^3H to ^{14}C decays

Experiment	Δt (h)	R_1 (ratio in vesicles)	R_2 (ratio in red cells)	R_2/R_1
1	0	120	80	0.67
2	0	69	48	0.69
3	0	68	51	0.75
1	20	120	87	0.72
2	20	69	52	0.75
4	20	240	163	0.68
2	43	69	50	0.72
Mean = $0.71 \pm .03$				

The third column gives this ratio (R_1) for the vesicles at the end of the first incubation. The fourth column gives this ratio (R_2) for the red cells at the end of a 2-h second incubation, which followed the first incubation after a delay Δt . See the text for experimental details. Ratios of ^3H to ^{14}C decays measured in red cell lipids and vesicles were determined in triplicate to within 5%. Error caused by the sticking of vesicles to red cells was determined separately and was found to be insignificant in our experimental conditions.

vesicles over a 120-h incubation period. Between 0 and 60 h the specific activity of the ^3H -cholesterol in the vesicle decreases from 14.9×10^5 d.p.m. per mg cholesterol to 4.3×10^5 d.p.m. per mg cholesterol representing a 71% loss in labelled cholesterol. The specific activity of the ghost cholesterol increases from zero to 0.3×10^5 d.p.m. per mg cholesterol, which is indicative of the large excess of cholesterol in the acceptor membrane. This 71% exchangeable and 29% non-exchangeable cholesterol pool in the vesicles correlates well with the evidence of Huang⁷ that 70% of the total membrane cholesterol is located on the outer half of the vesicle bilayer, and suggests that the cholesterol on the inner half of the bilayer is not accessible for exchange. The possibility that the non-exchangeable cholesterol observed in these experiments indicated the presence of inner bilayers in the vesicles is excluded, since quantitatively similar results were obtained using sized vesicles that had been chromatographed on a Sepharose 4B column by the method of Huang, and are homogeneous by established criteria. A tentative conclusion is that cholesterol does not flip from the inner half of the bilayer to the outer half within the time of the experiment.

In a further experiment, 1:0.9 DPL-cholesterol vesicles containing ^{14}C -lecithin but no ^3H -cholesterol were incubated for 8 h with red blood cells containing ^3H -cholesterol. The vesicles were then separated from the red cells and incubated with an excess of ghosts in the same manner as the previous experiment. Figure 1b shows that all of the ^3H -cholesterol can be exchanged from the vesicles in 24 h. This is consistent with the conclusion that the cholesterol exchanged into the vesicles from the red cells did not flip to the inner surface and therefore remained on the outside available for exchange with the acceptor membrane.

In a different set of experiments designed to compare the size of the exchangeable pool with the total cholesterol pool, vesicles were made with DPL and ^3H -cholesterol, and then ^{14}C -cholesterol was exchanged into the vesicles by incubating them with red cells containing ^{14}C -cholesterol for 4–5 h (first incubation). By introducing labelled cholesterol in these two ways, the ^3H -cholesterol will be found throughout the vesicle, whereas the ^{14}C -cholesterol will have been introduced only into regions of the vesicle reached by the exchange process. Following the first incubation the vesicles were isolated for a period Δt , after which the vesicles were incubated with unlabelled red cells to facilitate the exchange of cholesterol between the two systems (second incubation). The proportion of ^3H and ^{14}C present in the vesicles at the end of the first incubation and the proportion of the isotopes found in the red cells at the end of the second incubation were measured. The results of these experiments are given in Table 1. The ratio of ^3H to ^{14}C measured in the red cells was the same at the end of a second incubation of either one or two hours. Furthermore, this proportion was independent of Δt for values of this delay up to 43 h,

the longest time tested. It was found that the ratio of ^3H to ^{14}C had a value in the red cells which was 0.71 ± 0.03 of its value in the vesicles.

The finding that the ratio of ^3H to ^{14}C in the red cells was different from that in the vesicles means that the exchange process does not sample vesicle cholesterol uniformly, or that all of the vesicle cholesterol is not available for exchange. The time independence of the results indicates that the pool of cholesterol molecules which exchange with red cell cholesterol constitutes an isolated subpopulation of the total cholesterol in the vesicle. If this exchangeable pool represents the outer layer of molecules, then the isolation of this pool from the rest of the vesicle cholesterol would imply that there is no translocation of cholesterol between the two surfaces of the vesicle.

Let us denote the number of ^3H molecules in the exchangeable and non-exchangeable pools at the end of the first incubation by N_{H_1} and N_{H_2} respectively. All of the ^{14}C -cholesterol in the vesicles will be in the exchangeable pool, and we denote their number by N_{C_1} . The measured ratio of ^3H to ^{14}C decays in the vesicles at the end of the first incubation is: $R_1 = (N_{H_1} + N_{H_2}) / N_{C_1}$. The cholesterol exchanged into the red cells during the second incubation came from the vesicle exchange pool and the ratio of ^3H to ^{14}C decays in the red cell is therefore: $R_2 = N_{H_1} / N_{C_1}$. Algebraic manipulation yields $N_{H_2} / N_{H_1} = (R_1 - R_2) / R_2$ or $N_{H_1} / (N_{H_1} + N_{H_2}) = R_2 / R_1 = 0.71 \pm 0.03$.

In these experiments, the number of ^{14}C -cholesterol molecules in the vesicles was negligible compared with the number of ^3H -cholesterol molecules and therefore the value of 0.71 gives the fraction of vesicle cholesterol molecules which are accessible for exchange. As remarked before, this number is in good agreement with published values⁷ for the fraction of cholesterol molecules located at the outer surface of the DPL vesicle, and we conclude therefore that the exchangeable pool is the outer surface of the vesicle. That the exchangeable pool is isolated from the rest of the cholesterol in the vesicle is then the statement that cholesterol flip flop is extremely slow. Within the accuracy of our measurements, we can determine a lower bound for the half time to be 6 d. This lower bound was arrived at by taking values of 0.67 and 0.75 for the ratio R_2/R_1 for time delays of 0 and 20 h respectively, to fit an exponential process. This gives an extremely conservative estimate for the half time of flip flop. Similar rates of flip flop have been observed for phosphatidylcholine^{3,4}.

Slow cholesterol flip flop could have interesting implications for biological membranes. The slow rates observed for this process in vesicles may also occur in biological membranes which would explain the finding of some authors¹⁴ that there exists a pool of erythrocyte membrane cholesterol which is not exchangeable.

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Identification of β carbolines isolated from fluorescent human lens proteins

WITH ageing, a number of significant changes occur in the chemistry of the human lens. In the central region of the organ there is a slow transformation of protein to high molecular weight (HMW) aggregates greater than 50×10^6 (refs 1 and 2). It has been suggested that the relatively high concentration of such protein species in older lenses may cause significant light scattering^{3,4} and may be a contributing factor in the development of senile cataract characterised by central sclerosis and opacification⁵. An age-dependent increase in the insoluble protein fraction has also been observed⁶⁻⁸ and recent experiments indicate that the HMW species is an intermediate in the formation of this material¹⁵. Marked changes in the polypeptide chains of human lens proteins probably largely attributable to post-translational transformation, have also been observed¹⁶.

A yellowing of lens with age is also observed and its generation has been correlated with a concomitant increase in a protein bound non-tryptophan fluorescence⁸. This fluorescence is found primarily in the insoluble protein^{8,9} fraction of the nuclear region as well as with the HMW components of the soluble protein fraction⁸. It also has been shown that this fluorescence is associated with a 43,000 molecular weight polypeptide found in a number of HMW species and the insoluble fraction⁸. This polypeptide seems to increase in concentration with age and is not present in very young lens.

Alkaline degradation of either the insoluble fraction or HMW fractions in 4 N barium hydroxide at 110 °C for 24 h results in the release of a number of fluorescent components which have been separated and partially purified by preparative paper electrophoresis at pH 1.9. The principle fluorescent compound obtained by this procedure was designated 'C₁'. We report here the structure of this component and consider its relationship to the fluorescent material present in the lens proteins.

Approximately 1–2 g insoluble protein fraction obtained from 50–100 cataractous lenses were hydrolysed by 4 N barium hydroxide and C₁ was isolated by preparative paper electrophoresis at pH 1.9. Examination of C₁ by high pressure liquid chromatography using poragel PN in ethanol–water indicates that it contains considerable contamination and that only small amounts of it are present in the initial electrophoretically isolated preparations. C₁ has a yellow fluorescence when observed by long wavelength ultraviolet light (maximum 360 nm), is unstable and decomposes to two components characterised by a blue fluorescence designated Ia and Ib. These decomposition products have a much higher mobility towards the negative pole at pH 1.9 than their precursor C₁ and they are formed in all conditions including storage at 80 °C in the dry state. Oxidation of C₁ by potassium dichromate¹⁰ yields only two components which are the same as Ia and Ib on the basis of electrophoresis and fluorescent colour. Thin-layer chromatography (silica gel–EtOAc) indicated that Ia (r.f. = 0.20) was present in much greater amounts than Ib (r.f. = 0.25). Oxidation of the barium hydroxide hydrolysate of the insoluble protein yields two components which are the same as the products obtained from oxidation of C₁ on the basis of electrophoresis, thin-layer chromatography and fluorescence. Much higher yields of Ia and Ib are obtained by this procedure than through isolation and oxidation of C₁. This is undoubtedly attributable to the decomposition of C₁ which occurs during the hydrolysis and isolation procedures. A third fluorescent component was also observed. The origin, structure and significance of this third component are under investigation.

Studies on well characterised tryptophan-containing proteins such as bovine serum albumin failed to reveal the generation of any fluorescent components by alkaline hydrolysis¹⁰ using the methods described above. Furthermore, with such methodology, essentially no fluorescent components were found with either

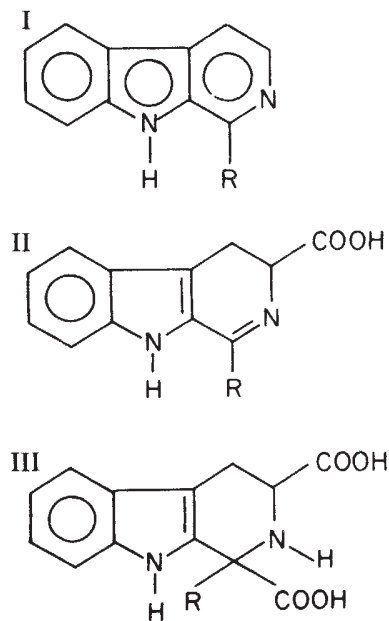
very young human lens proteins or calf lens protein. (Note that C₁ has been obtained by proteolytic digestion of succinylated, reduced and carboxymethylated protein from old human cataractous lenses⁸. Such protein is poorly digested and this approach is therefore not suitable for isolation and characterisation studies.) Hydrolysis of the above preparations in constant boiling hydrochloric acid¹¹, however, revealed significant amounts of components comparable with C₁. The amount of presumptive C₁ could be decreased but not eliminated by the presence of a reducing agent such as thioglycolic acid during the hydrolysis. Such observations suggest that C₁ was an alkaline hydrolysis product of precursors present in the insoluble lens protein and HMW fractions. In acid conditions, however, it was generated from normal tryptophan-containing proteins during the course of hydrolysis. The properties of C₁ and a consideration of the chemistry involved suggested that it might be a mixture of tryptophan derivatives, namely the 3,4-dihydro- β -carboline-3-carboxylic acids IIa and IIb (Fig. 1) and that Ia and Ib were fully aromatic β -carbolines. Synthesis^{10,12,13} of authentic Ia, Ib, IIa and IIb confirmed this hypothesis.

The following chromatographic and spectral data were obtained from both synthetic and natural Ia (about 15 μ g per 750 mg insoluble protein): thin-layer chromatography (silica gel–EtOAc), r.f. = 0.20; ultraviolet (in EtOH), 348, 335, 320s, 305, 287, 282, 267 and 250 nm; fluorescence (uncorrected, in EtOH), excitation at 350, 338, 290 and 257 nm, emission at 385 nm; mass spectrum, 182 (100%, M⁺), 181 (33%, M⁺–H), 154 (33%, M⁺–H–HCN), 127 (14%, M⁺–H–2HCN).

For synthetic and natural IB (about 7 μ g for 750 mg insoluble protein) the following chromatographic and spectral data were obtained: thin-layer chromatography (silica gel–EtOAc), r.f. = 0.25; ultraviolet (in MeOH), 349, 335, 288, 282, 250s nm; fluorescence (uncorrected, in EtOH) excitation at 350, 338, 290 and 257 nm, emission at 385 nm; mass spectrum, 168 (100% M⁺), 167 (10%, M⁺–H), 140 (35%, M⁺–H–HCN), 114 (18%, M⁺–2HCN), 113 (14%, M⁺–H–2HCN).

As stated earlier C₁ is unstable and furthermore, since the amount of C₁ isolated from the lens is very small, direct spectral studies of this material were very difficult. Comparisons of electrophoretic and chromatographic data of C₁ with those of a mixture of authentic 1-methyl-3,4-dihydro- β -carboline-3-carboxylic acid (IIa) and 3,4-dihydro- β -carboline-3-carboxylic

Fig. 1 β -carboline structures. I, Unsaturated β -carbolines; II, 3,4-dihydro- β -carboline-3-carboxylic acid; III, 1,2,3,4-tetrahydro- β -carboline-1,3-dicarboxylic acid. In the a form R = H; in the b form R = CH₃.



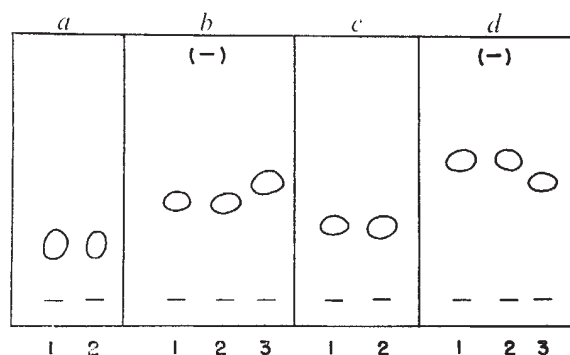


Fig. 2 *a*, Thin-layer chromatography comparison of (1) C_1 and (2) a mixture of Ila and Iib (cellulose-0.01% NH_4OH). *b*, Electrophoretic comparison of (1) C_1 , (2) a mixture of Ila and Iib and (3) tryptophan (pH 1.9, 2,500 V, 25 min). *c*, Thin-layer chromatography comparison of methyl esters of (1) C_1 and (2) Ila (silica gel-EtOAc). *d*, Electrophoretic comparison of (1) C_1 methyl ester, (2) Ila methyl ester and (3) tryptophan (pH 1.9, 2,500 V, 25 min).

acid (Iib) demonstrated that they consisted of the same components (Fig. 2*a* and *b*). Note that the oxidation of C_1 yields Ia and Ib, which are also obtained by oxidation of Ila and Iib, respectively. Ila and Iib on standing also decomposes to Ia and Ib with Iib being much less stable than Ila. Thus there is a disproportionate loss of Iib during the isolation of C_1 , which consequently explains the small amounts of Ib obtained from the oxidation of C_1 . C_1 and Ila had the same fluorescent characteristics (excitation at 335 nm, emission at 495 nm; uncorrected in 0.01% NH_4OH) and their methyl esters showed the same mobility on thin-layer chromatography and electrophoresis (Fig. 2*c* and *d*).

C_1 is probably a degradation product formed during the alkaline hydrolysis process. Therefore, three questions remain to be answered: Does the β -carboline ring structure exist *in vivo* in the lens protein? If so, in what oxidation state does it exist, and how is it linked to the lens proteins? We do not have direct evidence pertaining to the first question at this time, but the following experiment is suggestive. Protein from various sources were hydrolysed with base, oxidised with potassium dichromate, neutralised, extracted with ether and spotted on thin-layer chromatography. Protein obtained from 45- and 77-yr-old normal human lenses contained Ia and Ib. But significantly, essentially none of these components was detected in the protein of young human and calf lenses or bovine serum albumin. This suggests that there is an age-dependent formation of the β -carboline skeleton in human lenses. The second and third questions are more difficult to answer; however, preliminary experiments suggest that the *in vivo* precursor to the 3,4- β -carboline-3-carboxylic acids Ila and Iib are 1-methyl-1,2,3,4-tetrahydro- β -carboline-1,3-dicarboxylic acid (IIIa) and 1,2,3,4-tetrahydro- β -carboline, 1,3-dicarboxylic acid (IIIb) (Fig. 1).

Base hydrolyses of synthetic IIIa (ref. 12) indeed gave the 3,4-dihydro- β -carboline-3-carboxylic acid (Ila). These dicarboxylic acid structures are particularly attractive since they can act as a cross link between two polypeptide chains. Studies on the fluorescent 43,000 molecular weight polypeptide support this hypothesis. There is essentially no protein synthesis in the central region of the lens¹⁴. Thus the age-dependent appearance of the 43,000 molecular weight fluorescent polypeptide in this region must be attributable to the cross linking of two or more lower molecular weight components.

The mechanism by which the β -carbolines are formed in the lens is not understood at present. It is probable, however, that tryptophan is the precursor reacting with an unknown compound to form the β -carboline structure. This problem is now under investigation.

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Light-induced fast conformational change in all-trans-retinal at low temperature

AN understanding of the excited state properties of retinal is essential for the elucidation of the mechanism of the visual process, because the photochemistry of the visual chromophore accounts to a large extent for the early bleaching stages of rhodospin¹. All-trans-retinal has some unusual optical properties. It exhibits a dependence of its fluorescence quantum yield on the exciting wavelength^{2,3} as well as an anomalous heavy atom effect⁴. In addition, the nature of its emitting state has not been determined; there is evidence⁵⁻⁷, however, for the presence of a low lying, weakly allowed 1A_g state in some other polyenes.

I report here the nanosecond fluorescence properties of all-trans-retinal in a non-polar matrix at 100 K. Two samples of all-trans-retinal (Sigma and Eastman Kodak) were used with identical results. The purity of the samples was ascertained by thin-layer chromatography¹. A nanosecond fluorometer with a boxcar averager^{8,9} was used. A 7-60 Corning filter was used for excitation; the fluorescence quantum yield is constant² when exciting in the spectral region in which the filter transmits (maximum transmission is at ~ 360 nm).

Nanosecond time-resolved fluorescence spectra in a mixture of 1:1 by volume of isopentane and methylcyclohexane at 100 K are shown in Fig. 1. A broadening of the spectrum with time in the nanosecond range is observed. The full width to half maximum (FWHM) of the spectrum obtained at 10 ns after the peak of the exciting light pulse is 127 nm; that of the spectrum obtained at 1.5 ns before the peak of the exciting light pulse is 113 nm. But the spectral maximum as well as the long wavelength portion of the spectrum are invariant with time.

Figure 2 shows a strong dependence of the form of the decay on the emission wavelength. At 460 nm the decay is non-exponential; there is a long component corresponding to a decay time of about 3 ns at long times (small values of $W(t)$) and a much shorter component corresponding to a decay time of about 0.3 ns at short times. As the emission wavelength increases, the decay becomes faster but it continues to be non-exponential up to about 510 nm. At

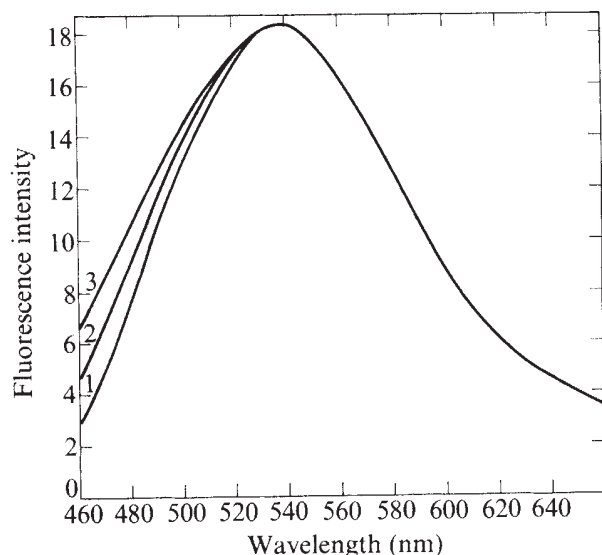


Fig. 1 Normalised time-resolved fluorescence spectra of a 10^{-5} M solution of all-*trans*-retinal in a mixture of 1:1 by volume of isopentane and methylcyclohexane at 100 K. The spectra were taken at the following times relative to the peak of the exciting light pulse (taken as zero time): (1) -1.5 ns; (2) +4 ns; (3) +10 ns. FWHM of the exciting light pulse ≈ 4 ns. A 7-60 Corning filter was used for excitation. The emission was analysed using a high intensity Bausch and Lomb scanning monochromator at a bandwidth of 19 nm. The spectra were not corrected for the variation of the photomultiplier-monochromator sensitivity with wavelength.

longer wavelengths the decay is virtually single exponential and independent of the emission wavelength; a decay time of about 0.1 ns is obtained in this wavelength region. It is significant that the variation of the form of the fluorescence decay with the emission wavelength occurs in the wavelength region for which the fluorescence spectrum is time dependent; this suggests that these two phenomena have a common origin. It should be noted that a decay time of

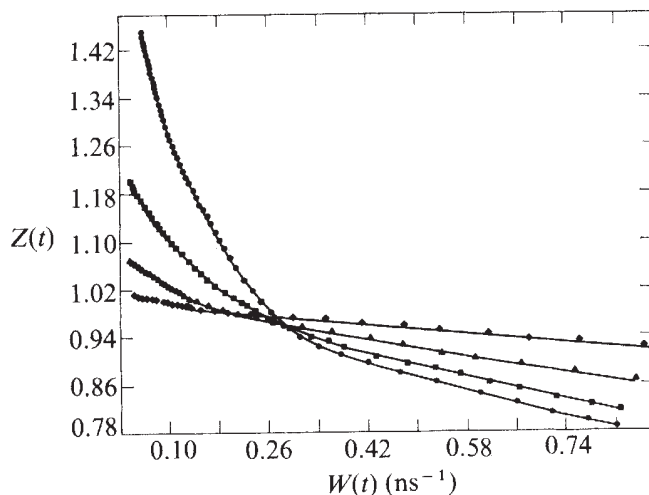


Fig. 2 Phase-plane plots for the fluorescence decay of a 10^{-5} M solution of all-*trans*-retinal in a mixture of 1:1 by volume of isopentane and methylcyclohexane at 100 K for the following emission wavelengths: $\bullet$, 460 nm; $\blacksquare$, 480 nm; $\blacktriangle$, 500 nm; $\blacklozenge$, 560 nm. The data were deconvoluted according to the method of Demas and Adamson¹². Long times correspond to small values of $W(t)$. For a single exponential decay a phase-plane plot yields a straight line of negative slope equal to the decay time. A 7-60 Corning filter was used for excitation. The emission wavelengths were selected by using a high intensity Bausch and Lomb monochromator at a bandwidth of 19 nm.

~ 0.6 ns has been reported⁴ in absolute ethanol at 77 K; this value apparently refers to the long wavelength region.

Calculations¹⁰ have shown that, as a result of electronic delocalisation brought about by excitation, the excited state-equilibrium torsional angle ϕ_{6-7} between the cyclohexene ring and the polyene chain of all-*trans*-retinal is very different from that in the ground state. I infer then, that immediately after light absorption, relaxation will occur from the Franck-Condon electronic state, which is associated with the ground-state conformation, to an equilibrium excited state associated with a modified conformation. I therefore attribute the time and emission wavelength-dependent phenomena reported here to a relaxation process in the excited state resulting from rotation of the polyene chain about the C6-C7 bond. The relaxation time for this process depends of course on the microviscosity of the molecular environment. The spectral broadening observed in the nanosecond range implies that, under my experimental conditions, the relaxation process competes efficiently with the deactivation of the excited state by radiative and radiationless processes.

The results of a picosecond study¹¹ indicated that the formation of prelumiherhodopsin when rhodopsin is irradiated probably involves a restricted change in the geometry of retinal rather than a complete isomerisation. The light-induced conformational change reported here may well be important in this first event of the visual process.

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Erratum

In the article "Persistence of CMV genome in lymphoid cells after congenital infection" by J. H. Joncas, J. Menezes and E.-S. Huang (*Nature*, **258**, 432; 1975) the correct name of the third author should be Eng-Shang Huang. In line 7 of the article C. H. Huang should read E.-S. Huang.

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matters arising

Algal sexuality

CAVALIER-SMITH¹ raises many interesting points on the origin of the eukaryotic cell and its organelles. I find myself in general agreement with him and with others²⁻⁴ who have provided an alternative to the widely held theory that eukaryotic organelles were originally acquired by endosymbiosis. I wish to comment, however, on one aspect of his evolutionary scheme (see ref. 1, Fig. 5), namely, the assertion that sex is absent in the Dinophyceae, Euglenophyceae, and Cryptophyceae. In fact, there have been several reports⁵⁻⁷ of sexuality in the Dinophyceae. Beam and Himes⁸ have observed pairing between cells of *Cryptocodinium cohnii* and their fusion into a single cell, followed by nuclear fusion. Furthermore, those workers⁸ and others⁹ have demonstrated genetic recombination in *C. cohnii*. Although there has been no such conclusive demonstration of sexuality in the Euglenophyceae, it is necessary to approach this negative evidence cautiously—especially since there is evidence of a meiotic process in two genera of this group^{10,11}. One can only repeat Leedale's careful conclusion that "It seems probable that there is no sexuality in the majority of euglenoids, but it remains a possibility that the process does occur as a rare phenomenon; this can never be disproved". Finally, there is a dearth of information on the Cryptophyceae, and consequently it is premature to conclude that sexuality is absent in that group.

These observations do not detract from Cavalier-Smith's main arguments. If anything they strengthen his evolutionary scheme since, if sex does exist in the Dinophyceae, Euglenophyceae, and Cryptophyceae, one could move those groups from their isolated position on Fig. 5 of ref. 1 to a position among the other algae and speculate on their interrelationships. (For example, how are the Dinophyceae and Cryptophyceae related to other algae whose chloroplasts contain chlorophylls *a* and *c*?)

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⁸ Beam, C. A., and Himes, M., *Nature*, **250**, 435-436 (1974).

⁹ Tuttle, R. C., and Loeblich, A. R., *Science*, **185**, 1061-1062 (1974).

¹⁰ Krichenbauer, H., *Archs Protistenk.*, **90**, 88-122 (1937).

¹¹ Leedale, G. F., *Archs Mikrobiol.*, **42**, 237-245 (1962).

¹² Leedale, G. F., in *The Biology of Euglena* (edit. by Buetow, D. E.), 185-242 (Academic, New York and London, 1968).

Irradiation and DNA breaks

JOHANSEN and Boye¹ reported their findings on the controversial question of whether the initial number of breaks induced by ionising radiation in DNA in oxic and anoxic conditions are identical, or whether less breaks occur in nitrogen than in oxygen. Their conclusion supports the latter.

They found¹ the difference in the yield of breaks in cellular DNA when *Escherichia coli* was irradiated in oxic and anoxic atmospheres and to minimise rapid repair² as far as possible, the time from irradiation to lysis of the cells was, according to those authors, "a fraction of a second". They assumed that cellular repair enzymes would not rejoin breaks in this short time and on the basis of some theoretical extrapolations on the correlation between the yield of breaks at a given dose and the rate of rejoining by cellular enzymes, they believed that the differences in yield of breaks due to rapid repair was an erroneous conclusion. They assumed that rejoining of breaks would not have occurred and if so, that the initial yield of breaks in oxic irradiation was at least three times more than that in anoxic conditions. I wish here to analyse the status of this controversy and I disagree with Johansen and Boye¹.

The subject has long been a matter of debate and Dean *et al.*³ were the first to suggest that anoxic breaks were subject to rapid enzymatic repair, showing that in experimental conditions which inhibited rejoining of strand breaks, nearly identical yields were obtained following oxic and anoxic irradiation.

The isolation of a DNA polymerase I mutant⁴ and the study of the kinetics of single-strand break repair in *polA1* mutant² led to the recognition of a rapid repair system occurring in *E. coli*. The work on cellular DNA involved complications because repair probably

works very fast, rejoining breaks during irradiation and up to the moment of lysis. Town *et al.*⁵ attempted to overcome the problem by preinactivating the cells at 52 °C for 10 min before irradiation and they obtained identical yields of breaks. Heat treatment, however, induces single-strand breaks in DNA⁶ and release of sulphhydryl compounds in the medium⁷.

Another approach⁷ was to irradiate bacteriophage λ extracellularly in oxic and anoxic atmospheres. Contrary to the situation for cellular DNA, any change in phage DNA would thus remain unmodified and beyond the influence of cellular repair as long as the DNA was not injected into a host. DNA could safely be extracted and layered on an alkaline sucrose gradient⁷. Identical yields of DNA breaks were obtained in oxic and anoxic extracellular irradiation⁷. When λ DNA was irradiated intracellularly in a *polA1* host, the decrease in molecular weight of λ DNA was identical in both oxic and anoxic conditions and equalled that obtained after extracellular irradiation. Intracellular anoxic irradiation of λ DNA to the same dose in wild-type bacteria, yielded no breaks⁷. This demonstrates that there is no difference in the initial number of breaks and that a rapid repair system, involving DNA polymerase I, rejoins breaks very rapidly. Similarly, there are data^{8,9} on the equal number of breaks in oxic and anoxic conditions with phage T3 and T7 irradiated extracellularly.

Wild-type bacteria were injected for 10 min with λ DNA which had been irradiated extracellularly with 50 krad in oxic and anoxic conditions. λ DNA was immediately analysed for single-strand breaks. λ DNA irradiated anoxically had no breaks, but a few could be seen in DNA irradiated in oxygen. Since the initial yields of breaks were identical⁷, the difference in molecular weight after infection is attributable to cellular repair.

Sulphydryl compounds may have a function in the radiation sensitivity of *E. coli*¹ but I feel that the experiments described (ref. 7 and above), supported by other results^{2,5,8,9} should leave no doubt that: (1) initially the number of DNA breaks are identical in oxic and anoxic irradiation and (2) rapid repair, involving DNA polymerase I and probably ligase, does exist in cells and preferentially rejoins anoxic-type breaks. This could explain the differ-

¹ Cavalier-Smith, T., *Nature*, **256**, 463-468 (1975).

² Allsopp, A., *New Phytol.*, **68**, 591-612 (1969).

³ Raff, R. A., and Mahler, H. R., *Science*, **177**, 575-582 (1972).

ences in the number of breaks in cellular DNA.

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¹ Johansen, I., and Boye, E., *Nature*, **225**, 740 (1975).

² Town, C. D., Smith, K. C., and Kaplan, H. S., *Science*, **172**, 851 (1971).

³ Dean, C. T., Ormerod, M. G., Seriani, R. W., and Alexander, P., *Nature*, **222**, 1042 (1969).

⁴ De Lucia, P., and Cairns, J., *Nature*, **224**, 1164 (1969).

⁵ Town, C. D., Smith, K. C., and Kaplan, H. S., *Radiat. Res.*, **52**, 99 (1972).

⁶ Woodcock, E., and Grigg, G. W., *Nature new Biol.*, **237**, 76 (1972).

⁷ Srivastava, B. S., *Int. J. Radiat. Biol.*, **26**, 391 (1974).

⁸ Freifelder, D., *Radiat. Res.*, **29**, 329 (1966).

⁹ Neary, G. J., Simpson-Gildemeister, V. F. W., and Peacocke, A. R., *Int. J. Radiat. Biol.*, **18**, 25 (1970).

JOHANSEN AND BOYE REPLY—We believe Srivastava's conclusions¹ are based on misinterpretations of circumstantial evidence. First, it has long been known that when bacteriophages are irradiated extracellularly the survival of their plaque-forming ability is identical in oxygen and in nitrogen. When irradiated in the presence of sulphhydryls, however, the survival in nitrogen is increased, and becomes higher than in the presence of oxygen². This suggests the importance of sulphhydryl compounds in anoxic protection. The finding that the yield of DNA strand breaks is similar in oxic and anoxic conditions when phage λ is irradiated extracellularly in the absence of sulphhydryls³ is as expected, and within the framework of classical radiation biology.

Second, when irradiated intracellularly, in normal physiological condi-

bacteria heat shocked before irradiation⁴; (3) bacteria kept at pH 8.6 (ref. 8). During these treatments, the cells become leaky and considerable amounts of sulphhydryls are lost into the suspending medium⁵; (4) Srivastava's own experiment with intracellular phage λ (ref. 3). We noticed that in this experiment the bacteria were suspended in 0.01 M MgSO₄ before irradiation, and wondered whether this hypotonic treatment had any effect on the level of endogenous sulphhydryls in the bacteria. As can be seen from Table 1, suspending bacteria in 0.01 M MgSO₄ leads to a loss of a considerable amount of sulphhydryls from the cells. Small contaminating amounts of oxygen—if present—will increase the 'anoxic' yield of radiation-induced DNA strand breaks in these cells.

Third, experiments in which phage λ is irradiated extracellularly in oxygen or in nitrogen in the absence of sulphhydryls, and the DNA analysed 10 min after infection of a repair-proficient strain are very complex, and interpretation is not unambiguous. In conclusion, recent experiments^{4,5} tend to support the view that the oxygen effect in radiation-induced DNA strand breakage is due to radiochemical reactions, rather than to preferential enzymic repair.

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¹ Srivastava, B. S., *Nature*, **259**, 425–426 (1976).

² Howard-Flanders, P., *Nature*, **186**, 485 (1960).

³ Srivastava, B. S., *Int. J. Radiat. Biol.*, **26**, 391 (1974).

⁴ Johansen, I., Brstad, T., and Rupp, W. D., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 167 (1975).

Table 1 Effect of hypotonic treatment on cellular sulphhydryls

Treatment*	Sulphhydryls in cell-free supernatant (mM)	Sulphhydryls in TCA extract (mM)
Phosphate buffer, pH 6.8	<0.05	2.04
0.01 M MgSO ₄	1.15	1.12

*Cells of *E. coli* K12, strain AB 1157, from a 100-ml log-phase suspension were collected by centrifugation and washed in phosphate buffer¹ at pH 6.8. The cells were resuspended in 0.3 ml of the same buffer or in 0.3 ml 0.01 M MgSO₄, kept for 10 min at room temperature and the concentration of sulphhydryls measured in the cell-free supernatant⁵. The bacteria were suspended in 0.3 ml trichloroacetic acid (TCA) at 0.3 M, kept at room temperature for 10 min and the concentration of acid soluble sulphhydryls measured in the cell-free extract.

tions, a higher yield of strand breaks is generally found in oxic than in anoxic conditions, both for chromosomal and phage DNA. This is true even when the experiments are performed so fast that the ligase could rejoin, at the most, one of a hundred DNA strand breaks formed^{4,5}. We are aware of four types of experiment where the anoxic yield of DNA strand breaks is increased to—or nearly to—the oxic yield: (1) bacteria treated with the sulphhydryl-binding agent *N*-ethyl-maleimide (NEM) before irradiation⁶. This sensitisation is reversed by strict anoxia⁷ and is believed to be caused by increased sensitivity to small contaminating amounts of oxygen in cells with low levels of endogenous sulphhydryls⁸; (2)

size or development^{1,2}. Chang *et al.*² investigated the well-known asymmetry of the scrotum in man and showed that in right-handed subjects the right testis tended to be higher, whereas the converse applied in left-handed subjects. To investigate whether this was simply due to the greater weight of the left testis in right-handed subjects they measured the weight and volume of the testes in (presumably mainly right-handed) cadavers and found, paradoxically, that the right (that is, the higher) testicle was also the heavier and of greater volume, a result in accord with Mittwoch and Kirk's foetal data¹.

Interest in testicular asymmetry may however be traced back much further. Winckelmann³ in 1764 commented that: "Even the private parts have their appropriate beauty. The left testicle is always the larger, as it is in nature;". He went on, however, "so likewise it has been observed that the sight of the left eye is keener than the right", an observation which, to my knowledge, has not been confirmed.

To test Winckelmann's claim, I observed the scrotal asymmetry of 107 sculptures, either of antique origin or Renaissance copies, in a number of Italian museums and galleries. Table 1 shows that although the ancient artists were correct in tending to place the right testicle higher, they were wrong in so far as they also tended to make the lower testicle the larger: we may postulate that they were also using the common-sense view that the heavier ought to be lower. Although Winckelmann's observations of antique sculpture were correct, his observations of nature are clearly in error.

The reason for the artists placing the right testicle higher than the left is not clear. It may reflect the true observed state of things, but it may also be a function of Greek left/right symbolism, in which right and male, and left and female were regarded as equivalent, and thus for instance, the male child was presumed to come from the right (and thus higher?) testis, and vice versa for the female child⁴.

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¹ Mittwoch, U., and Kirk, D., *Nature*, **257**, 791–792 (1975).

² Chang, K. S. F., *et al.*, *J. Anat.*, **94**, 543–548 (1960).

³ Winckelmann, J. J., in *History of Ancient Art* (transl. by Gode, A.) (Frederick Ungar, New York, 1968).

⁴ Lloyd, G. E. R., *J. Hellenic Stud.*, **82**, 56–66 (1962).

Scrotal asymmetry in man and in ancient sculpture

MITTWOCH and Kirk¹ have claimed that "Right and left mammalian gonads do not usually differ noticeably either in

Table 1 Analysis of the scrotal asymmetry of 107 ancient sculptures

		Side of higher testicle			Total
		Left	Equal	Right	
Side of larger testicle	Left	2	7	32	41
	Equal	8	19	17	44
	Right	17	1	4	22
	Total	27	27	53	107

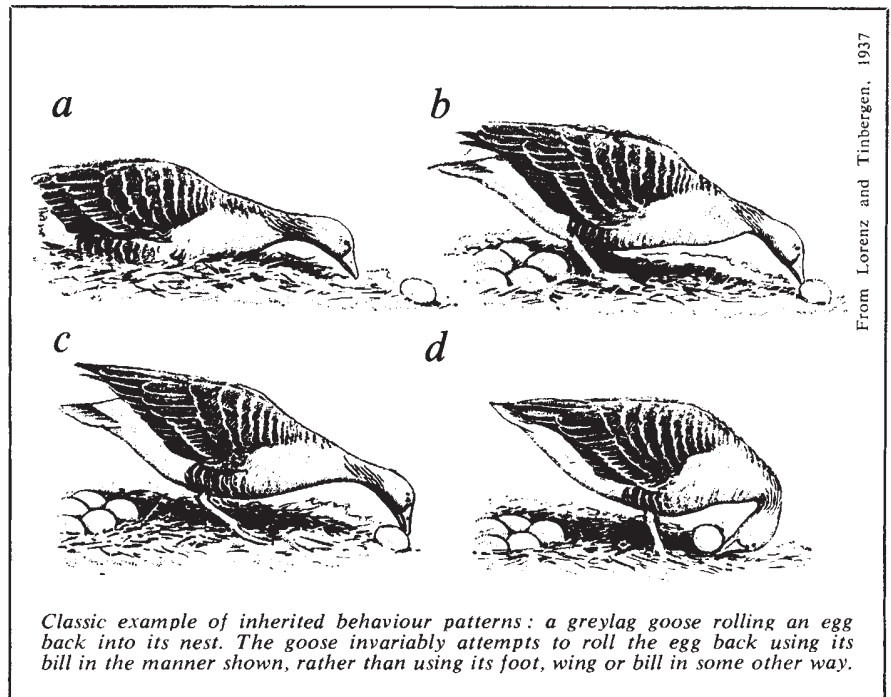
reviews

Optimum strategy for perpetuating genes?

ANY textbook on behaviour which attempts to go beyond the single topic monograph, presents its author with formidable problems concerning the selection and organisation of material. Behavioural studies may have their origins in fields as far separated as neurophysiology and ecology. Brown's book* has evolution as a unifying theme, and indeed behaviour itself is not always its main focus, for it contains a great deal of material pertinent to the mechanisms of evolution which would not normally be found in a behavioural text. It is very much a product of the new sociobiology school, and none the worse for that. We have certainly gained many fresh insights into animal behaviour from a consideration of the selective forces which operate on individuals with respect to their interactions with others of their species.

Brown operates very skilfully on the borderlines between ethology, population biology and genetics, and he is highly competent in all of them. His selection of literature is good and experimental results are described, and often illustrated, in sufficient detail for the reader to understand their significance fully. On those topics where I am competent to judge the adequacy with which a controversy is being discussed, Brown misses very few of the points and this is a rare quality in textbooks.

There are some penalties to be paid for being so comprehensive. The book is very long and the choice of more purely behavioural topics for inclusion seems rather arbitrary. Brown begins with a brief prologue which I found rather odd. He lays stress on diversity between species and constancy within species without once mentioning change. Indeed almost at the outset he states that species "... tend to persist



From Lorenz and Tinbergen, 1937

Classic example of inherited behaviour patterns: a greylag goose rolling an egg back into its nest. The goose invariably attempts to roll the egg back using its bill in the manner shown, rather than using its foot, wing or bill in some other way.

virtually the same for generation after generation." True, but not an idea I would choose to emphasise at the start of a book on evolution. The changes which natural selection can elicit from populations form the bulk of the book which follows.

The book is organised into six sections of very diverse proportions. Thus the first two on phylogeny and genetic basis of behaviour have but single chapters. The former is a brief but fresh survey of the possibilities for analyses of the phyletic descent of behaviour within and between groups. The genetics chapter is less satisfactory, although it provides a reasonable review of the different approaches in behaviour genetics (with however, no reference to the quantitative analysis of the genetic architecture of behavioural traits). Nevertheless this chapter seems very isolated from the rest of the book because our knowledge of the inheritance of behaviour does not yet match up to some of the questions which evolutionary studies force us to ask.

The book's real qualities emerge in sections 3 and 4 on social organisation and communication. Brown gives an excellent account of the modern sociobiological approach to animals in groups. He deals with the factors which determine the nature and size of groups and goes on to discuss mating systems and aid-giving behaviour of all

types. He presents most of the arguments very clearly in depth and I find it the more strange that Wynne-Edwards' ideas on group selection and the origins of social behaviour are given such brief treatment. They seem to me of great importance not because they are right (I think they clearly are not) but because of the effect they had on the course of sociobiology. The controversy which surrounded Wynne-Edwards' theories led to a great deal more attention being focused on the individuals within the group. This was vital in the development of the modern synthesis of behavioural and evolutionary concepts.

Will all social behaviour, including that which gives aid to others, turn out to be an optimum strategy for perpetuating one's genes? Certainly we need to know as much as possible about the genetic relationships within groups. Brown provides a very good review of the evolution of behaviour in relation to kin selection. He manages to retain clarity on some complex issues without oversimplifying.

The section on communication is more purely behavioural but the material provides some of the classic stories in the evolution of behaviour. Just as did Marler and Hamilton in their 1967 textbook, so Brown by skilful choice of examples manages to make the whole subject fresh. There is also a

**The Evolution of Behaviour*. By Jerram L. Brown. Pp. xix+761. (W. W. Norton: New York, 1975.) \$15.95.

Good but expensive fare

Introduction to Physiology. By Hugh Davson and M. B. Segal. Volume 1: Basic Mechanisms, Part 1. Pp. xii+561. Volume 2: Basic Mechanisms, Part 2. Pp. xi+481. (Academic: London and New York, June 1975.) £6.80 each part.

THESE two volumes are the first to appear in a series of five. A reviewer thus faces a similar dilemma to a diner passing judgement on a complete meal after sampling only the soup and fish. In this instance, however, the renown of the chef de cuisine, Dr Hugh Davson, is such that it is only necessary to decide for what type of clientele he is catering to predict what is in store. It turns out that, along with his co-author, Dr Segal, he is providing good, solid and easily digestible fare without too many sophisticated trimmings but also without succumbing to the hazards of oversimplification.

Volume 1 deals, in the opening chapters, with cellular physiology and includes a good deal of material usually classed as biochemistry—for example, the tricarboxylic acid cycle and protein synthesis. Such fundamental topics in general physiology as osmosis and the genesis of bioelectric potentials are also covered and up-to-date descriptions of ultrastructural findings related to cellular function and interactions are provided. Some elementary thermodynamics is also included but, although simple equations are used, the approach is essentially descriptive. The concept of chemical potential, although it receives a passing mention, is not seriously developed and the section on

entropy is one of the few instances in which the exposition is a trifle confusing, if not actually misleading. The remaining chapters are devoted to haemodynamics, respiratory exchange, in testinal absorption and renal function, all of which are treated in considerable detail. The second volume is concerned with the nervous system (chiefly the peripheral and spinal divisions), hormonal control (discussed in rather general terms), the effector systems (muscles and glands), defence mechanisms (mainly haematology and immunology) and reproduction (including some 20 pages on lactation).

The overall presentation is clear and commendably free from errors, although an occasional slip such as the heading "carbonate" over a section evidently devoted to carbamate could be tiresome to a beginner. The illustrations are of high quality and generally well chosen, but it seems odd to reproduce a typical Gamble diagram and attribute it to a recent review, and to opt for an oxygen dissociation curve published in 1958 which adds little to the one published by Bohr and Krogh in 1904. Students with hardly more scientific preparation than a nodding acquaintance with chemical formulae and graphs, should find these books comprehensible. After mastering the five volumes (if the succeeding ones resemble the first two) such students will be not only introduced to but thoroughly at home in physiology. Some, however, may find this protracted initiation and the entrance fee (presumably in five instalments of £6.80 each), which goes with it, too disheartening and will feel tempted to seek another portal of entry to the subject.

R. V. Coxon

very good account of the evolution of display movements although the motivational analysis of displays which has played such a large part in the development of this area of ethology, and which has strong evolutionary implications, is mentioned only in brief.

For the final sections of his book Brown chooses some topics on what he calls "the physiological basis of species constancy and species diversity in behaviour." The chapters on reflexes and fixed action patterns do fit the plan, but he goes on to deal with sensory systems, migration and circadian rhythms. They are all good, modern accounts, but I fail to see where they fit into Brown's evolutionary scheme. I have similar problems with the contents of the final section on development, in which the behaviour of embryos is covered in detail, but there is no mention of learning. The concluding chapter on bird song, however, does provide an opportunity to discuss the evolution of

development itself. The birds provide such a good range of ecological and social variants which we can now begin to correlate with the manner in which their song develops.

So this review ends as it began, quibbling about the selection of material. Brown's evolutionary theme has sufficient strength to give a solid backbone to his chapters even though all of us would leave some things out and include others. I think this is a distinguished textbook. In Britain it is probably too long for most undergraduate courses, but it can nevertheless unhesitatingly be commended as a book for students to consult. I defy anyone to browse in the central chapters without having their attention riveted by some elegant and fascinating example of the manner in which their behaviour has come to equip animals for survival and I can pay Brown no higher compliment.

Aubrey Manning

Cryogenics

Heat Transfer at Low Temperatures. (The International Cryogenics Monograph Series.) Edited by Walter Frost. Pp. xiv+382. (Plenum: New York and London, 1975.) \$42.00.

THIS book "is intended to enhance the knowledge of the thermal design engineer faced with solving heat transfer and fluid flow problems at low temperatures." The temperature range which is covered lies, in effect, between room temperature and 1K: thermal transfer problems peculiar to lower temperatures than this are not described at all. The book consists of 15 chapters, in reality separate articles prepared by 20 different contributors.

The first two chapters deal effectively with 'classical' thermal transfer through conduction and convection. The second, and main, part of the book consists of ten chapters devoted entirely to two-phase phenomena of various sorts. Topics such as nucleate pool boiling, film boiling, two-phase flow, and the condensation of gases on cryogenic surfaces are treated in considerable detail. The third part consists of two chapters on topics which, presumably, did not seem to the editor to fit in conveniently anywhere else: one on radiative heating, with particular reference to the influence of cryodeposits on the cold surface; and a final chapter on thermal transfer to HeII.

No attempt has been made to standardise nomenclature or units between the different chapters: pounds and feet coexist uneasily with kilograms and centimeters; BTU with ergs and joules; and Kelvins with degrees Centigrade and even Fahrenheit. The remark on the dustcover about "surveying the literature to date . . ." was no doubt accurate at the time of writing. While three or more years may perhaps be a fairly normal publishers' gestation period these days, it seems rather a pity that more could not have been done to update the book at the proof stage: this would, at least, have enabled some modification of the amusingly anachronistic remark in the final chapter to the effect that liquid 'He and the superconducting electron gas are the only known superfluids.

The book does contain a great deal of information, however, so that, in spite of these minor shortcomings, it should certainly be useful to aspiring designers of rockets, space simulators, superconducting generators and other large scale cryogenic machines involving the flow or storage of liquified gases.

P. V. E. McClintock

Molecular rearrangements

Isotopes in Organic Chemistry. Volume 1: Isotopes in Molecular Rearrangements. Edited by E. Buncl and C. C. Lee. Pp.xvi+301. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.100; \$41.75.

LABELLING experiments have often been crucial to the development and understanding of modern organic chemistry and few will question after reading this book that many mechanistic studies would be considerably harder, if not impossible, without the availability of isotopic techniques. Nonetheless, good texts and reviews of this field have been thin on the ground; this series, of which the pre-

sent volume is the first, should fill an important gap in the chemical literature.

Both carbonium ion (26 pages) and carbanion (105 pages) rearrangements are given another airing by N. C. Deno and D. H. Hunter, respectively, and pericyclic reactions (33 pages) are reviewed by W. R. Dolbier. J. S. Swenton's contribution concerns photochemically initiated rearrangements (51 pages) and J. L. Holmes deals with rearrangements occurring alongside mass spectral fragmentations (73 pages). As one might expect from these particular authors, of whom all have made significant contributions to their specialised topics, their articles are well-written from an authoritative standpoint, and all seem to cover the literature up to 1973. Generally, the

authors have written a fairly descriptive text of the kind usually appreciated by organic chemists and have attempted (with varying success) to relate the isotopic results to other mechanistic criteria. The editors have avoided the obvious pitfall of allowing repetitious theoretical introductions to each chapter, so the amount of worthwhile information is high.

This book (and probably the series, too) is written for the specialist with a deep interest in organic mechanistic studies. Elsevier might seriously have considered making each chapter available separately, particularly as the contents of the present volume span such a wide range of interests. Nonetheless, I strongly recommend the text as a valuable addition to any chemical library.

B. C. Challis



Illustrations taken from British Botanical and Horticultural Literature before 1800. By Blanche Henrey. Three Volumes. Pp. 1,128. 32 colour plates, 162 pp. black and white illustrations. (Oxford University: London, 1975.) £70. A comprehensive account of books and pamphlets on botany, gardening, horticulture, arboriculture and silviculture from the sixteenth to eighteenth centuries. Left, Helleborus niger L., Christmas Rose. Drawn and engraved by James Sowerby. From Medical Botany (1790), Vol. 1 by Woodville. Right, Lillium martagon L., Martagon or Mountain Lilly. Engraving from The Compleat Florist (1740).



Neoplastic development

Neoplastic Development. Volume 2. By Leslie Foulds. Pp. xiv+729. (Academic London and New York, August 1975). £16.80; \$43.50.

LESLIE FOULDS spent most of his working life in experimental cancer research, but his interest in cancer extended beyond the laboratory. His object in writing this book is perhaps best given in his own words:

In any branch of science it is important to confront inferences from laboratory experiments with the stark realities of natural phenomena... It is especially important... when the growing estrangement between clinical practice and laboratory research is leading to grave doubts about the ability of laboratory research, as now conducted, to make any substantial contribution to the alleviation of human suffering attributable to neoplastic disease. Writing from the standpoint of a medically qualified experimental pathologist with a long-standing interest

in both developmental biology and medical practice but with no direct participation in either, I hope to illustrate in this volume (Volume 2) the complimentary and essential, but diverse contributions, of biological theory, laboratory analysis and clinical experience of human disease...

Volume 1 provided a general theory, basically that neoplasia is a developmental process akin to normal development but differing from it in some important respects, still to be defined. In Volume 2, he considers special cases of neoplastic development in man, and animals, and discusses the biological and medical implications. After a brief general introduction, there are large sections on neoplasia of the skin, mammary neoplasia in laboratory animals and human breast cancer. Shorter sections deal with cancer of uterine cervix, urinary tract, lungs, liver and some endocrine glands, and there are two brief sections on neoplasia of unknown pathogenesis and virus tumours. The section on skin is the most extensive and is used to establish general

principle concerned with the initiation-promotion theory and with tumour progression. The latter is illustrated by descriptions of the disease process in man and in the experimental animal, and a discussion on aetiology. A similar approach is used in the other sections.

Like its predecessor it is a valuable work and few of those interested in cancer in general or in one of the special fields discussed will fail to learn something of value. It will teach the clinician something of experimental cancer research and the research worker a little about human cancer, but in a major objective—a more rational approach to the clinical management of the patient—it must be regarded as a gallant failure, not through a fault of the author but through our ignorance. It is sad that the author should have died from cancer shortly before the book was published, and before he completed the final chapter summarising his views and general conclusions.

L. M. Franks

Bold perspective

Ion-selective Electrodes. (Cambridge Monographs in Physical Chemistry Volume 2.) By Jiri Koryta. Pp. 207. (Cambridge University: Cambridge and London, August 1975.) £10.50.

THE development and application of ion-selective electrodes (ISEs) based on membrane electrochemical principles have been, with comparable activity in liquid chromatography, the two most rapidly advancing areas in chemical analysis and continuous monitoring. Since 1965 improved or new measurements have become possible which have not only given impetus to analytical potentiometry, but have a broad impact in diverse areas as environmental science, physiology, clinical chemistry and biophysics. In fact one difficulty in following progress in the field is the widespread use of ISEs

and the large number of specialist journals in which results appear. ISEs are serving the fundamental purposes of making analytical chemistry visible in other branches of chemistry, physics, medical and health sciences and the converse effect of broadening the interests of analytical chemists in interdisciplinary problems and projects.

To write a book at midstream in a rapidly moving field requires courage, and to achieve a sense of perspective on the past and possible future developments is most unusual. Koryta's new volume is well organised and executed, and contains clearly presented fundamentals and applications drawn from all fields touched by ISEs. Jiri Koryta is a well known electrochemist, a major disciple of Nobel Laureate J. Heyrovsky and an exponent of the polarographic method. His outstanding contributions to theory and practice of coupled homogeneous-heterogeneous electrode processes, complex ion equilibria and adsorption effects make him an ideal commentator on the field of ISEs and membrane processes. The primary strength of the book is the mature overview given on the topics of diffusion-migration and the interfacial processes which underlie the potential sources for fixed site, mobile site and neutral carrier classes of membrane electrodes. At the same time practical, analytical methods are tabulated and current topics such as continuous monitoring and use of computer-controlled titrations are described.

Only on hindsight can one determine key, definitive volumes on a given speciality area. Because of the continuing growth in this field, all previous books, although useful for reference, are clearly premature. This new book unfortunately must suffer from the same limitations: new fundamental studies on response mechanisms, time-dependence of responses and applications are still appearing in current literature at a rapid rate. This volume contains literature citations only as recent as 1973 and is an expansion of Koryta's excellent 1972 review article. Nevertheless, this book is the finest, comprehensive work published to date.

Richard P. Buck

Climatic change

Ice Ages: Ancient and Modern. (Proceedings of the 21st Inter-University Congress, January 1974.) Edited by A. E. Wright and F. Mosley. Pp. 320. (Seel House: Liverpool, 1975.) £12.00; \$33.00.

THIS book is advertised as suitable for geology and geography students "from sixth form to final year undergraduate level"; but unless the sixth formers of

today are markedly more able than those of my generation it would seem better suited to students of Earth sciences at a rather later stage of their academic careers, including those active in research. For such people, the book will be of most value to those who have had a less formal education in geography and geology at an earlier stage. It provides a good quick guide to geology and timescales, to processes of sedimentation and to glaciectonic structures, before moving on to discuss climate and climatic change in more detail.

There is one exceptionable statement early in the first section ("Introduction to the Quaternary") in which F. W. Shotton refers to ice ages occurring when "... snowlines extend outwards from the poles, ice sheets expand, mountain glaciers push forward ..." and makes no reference to the recent work which suggests strongly that ice ages can occur much more quickly than this older picture suggests, with snow and ice cover building up over a wide area of the northern hemisphere in a few years or decades. Otherwise, the various contributions (which are based on lectures given at Birmingham University in January 1974) provide a good overview of how geological and other evidence (pollen analysis, studies of fossil beetles and so on) provides an indication of how climate has fluctuated.

But the discussion of the causes of such changes is less satisfactory, and the volume also suffers from being almost entirely concerned with events in north-western Europe, particularly Britain. It is inexcusable, in a book of this length, to dismiss variations caused by changes in the Earth's orbit and inclination in less than a page and by volcanic dust in three lines. There is a preponderance of geological jargon in some of the contributions, and most of the contributors share the geologists' preference for talking in terms of the named geological periods of time, rather than in years b.p. These deficiencies are balanced to some extent by the copious references and by a summary from the editors putting the various contributions into perspective. In this case, the summary is particularly valuable and does not become bogged down by attempts at verbatim reporting of question and answer sessions. The importance of this kind of research, and the slightly parochial inclination of this book, are highlighted by one titbit from the summary: "even today it would only require an average temperature of 2 °C to [produce] glaciers on Ben Nevis".

Those searching for more answers in the study of climatic changes will be stimulated by this volume.

John Gribbin

Nuclear structure

Structure of the Nucleus. By M. A. Preston and R. K. Bhaduri. Pp. xiv+693. (Addison-Wesley: Reading, Massachusetts and London, August 1975.) Cloth \$29.50; Paper \$19.50.

FOR more than a decade Preston's *Physics of the Nucleus* has been used by nuclear physicists as a comprehensive and reliable reference book. Its essential theme is the attempt to derive the properties of the nucleus from our knowledge of the nucleon-nucleon forces. This can be done to some extent, but in many respects we still have to rely on phenomenological models. The years since 1962 have seen many important advances, and while progress has been made in some areas the problem is now seen to be more complicated than was previously thought, particularly because of the importance of many-body forces. To deal with this situation Professor Preston, now joined by Dr. Bhaduri, has thoroughly revised and re-written the chapters dealing with nuclear structure, and since this is already as long as the whole book before revision the chapters on nuclear reactions have been omitted. The plan is very similar and successive chapters deal with the constituents of nuclei, internucleon forces, nuclear moments, nuclear shapes and sizes, and nuclear binding energies. The second part is devoted to nuclear models, correlation in nuclear matter, collective nuclear motion, and Hartree-Fock and particle-hole calculations. The final part covers α radioactivity and fission. The standard of the original volume is amply maintained, and the new version will be a valuable and much-used text for years to come. **P. E. Hodgson**

Human tumour lines

Human Tumour Cells in Vitro. Edited by Jørgen Fogh. Pp. xix+557. (Plenum: New York and London, 1975.) \$44.50.

THIS book, a collection of 16 papers on human tumour cell culture, has appeared at an appropriate time. Belatedly research laboratories are beginning to show an increasing interest in cancer in man and at the same time there is an increasing, if somewhat misguided, pressure from society to limit suffering in laboratory animals, particularly since it cannot or will not limit suffering among men. Consequently *in vitro* systems, especially those using human tissue, are becoming increasingly popular. As in any branch of science the value of a particular technique depends on the questions it is being used to answer. If cell cultures are being used for virus production or cytotoxicity testing (for example), the nature of the cells is unimportant provided they serve the purpose of the experiment. If the experiments are concerned with the nature and behaviour of the cells themselves a more critical approach is required.

It is important to know the precise origin of the cells and the variations in structure and function which they may have developed as a consequence of establishment *in vitro*. This was well known to the early practitioners of the art of cell culture—all of whom were biologists or physicians accustomed to studying the whole individual—but with the expansion of cell and molecular biology, concerned principally with isolated cells, and an era when living cells could be ordered and supplied 'off the shelf' by laboratory supply houses, these basic facts were often forgotten. Although the whole universe may be contained in a grain of sand the whole of biology is not contained in a HeLa or BHK cell. Why for example is it possible to maintain such a small proportion of tumour cells in culture as permanently established cell lines? How can tumour cells *in vitro* be identified with certainty and distinguished from mesenchymal cells arising from the non-malignant stroma of the tumour? *Human Tumour Cells in Vitro* considers some of these problems.

Apart from a valuable description of 31 new human tumour lines by the editor and Dr Trempe, most of the information is available elsewhere to those with access to a good library but for the others it provides a useful survey of methods and media which have been used for the establishment of human tumour cell lines *in vitro*. There are also sections on cytological characterisation, chromosome abnormalities and

ultrastructure and on the search for viruses in human cancer cells.

L. M. Franks

Pineal physiology

Frontiers of Pineal Physiology. Edited by Mark D. Altschule. Pp. xii+269. (MIT: Cambridge, Massachusetts and London, 1975.) £8.75.

It would be a pity if this book were read as an entrée to the new and exciting realm of pineal physiology; its title stakes a claim which is wholly unjustified by its contents, a collection of papers (some reviews, others research reports, yet others an uneasy mixture of the two) uneven both in length and quality. Five years have passed since the conference on which it is based was held, and this latent period is all too obvious in places.

The intense enzymatic activity of the pineal, and the way this is changed by either natural or experimental lighting are characteristic features. Three chapters survey this area, with varying degrees of thoroughness. A firmer editorial hand might have improved the organisation of this part of the book, and reduced the considerable overlap between individual chapters; much of these have also appeared in other reviews by the same authors.

Although studies on pineal function are still dominated by the discovery of melatonin, increasing attention is now being given to pineal polypeptides, some of which seem to resemble those found in the hypothalamus and pituitary; two research reports deal with these substances. P. H. Sampson contributes a brief survey on the way the pineal may be involved in various categories of behaviour, most of which is a lengthy report of an experiment showing that pinealectomy somewhat reduced maternal behaviour. We are left wondering what this might mean in terms of pineal function, for no interpretation is offered.

The most intriguing, and inevitably the most controversial, part of the book explores the possible association between malfunction of the pineal and schizophrenia. The large amount of 5-hydroxytryptamine in the pineal and the suspicion that this monoamine is involved in schizophrenia make the link tempting (although drugs ameliorating this condition are generally dopamine blockers). M. D. Altschule contributes a chapter which is too brief either to inform adequately or to convince in which it is said that administering pineal extracts improves abnormalities in carbohydrate metabolism in schizophrenics, although how these are related to the disease is unclear. L. B. Bigelow describes the results of a trial in which schizo-

phrenics were given daily injections of melatonin-free pineal extract. In spite of a curious method of measuring the patient's mental state, an uneven baseline and coincident changes in phenothiazines, the results of at least one case suggest the need for a more comprehensive study. Dr Bigelow quotes the isolation from the pineal of a substance inhibiting the formation of dimethyltryptamines, long known to be hallucinogenic. What a pity his study didn't concentrate specifically on these symptoms.

This book represents the frontiers of our knowledge indistinctly, and the signposts to the future are hard to discern from reading it. J. Herbert

Xylem strategy

Ecological Strategies of Xylem Evolution. By Sherwin Carlquist. Pp. xi+259. (University of California: Berkeley, Los Angeles and London, August 1975.) £6.85.

THIS book is profoundly disappointing. The subject matter does have general interest. Do such features as the lengths of tracheids and vessel elements, the shapes and sizes of these cells in transverse section and the shapes and sizes of the pits between these cells have any significance in the adaptation of plants to their habitats? Is the disposition of parenchyma and fibres within and around the xylem of adaptive significance? Any satisfying book would have to start from a statement of theory regarding the functional effects of these features, together with such experimental evidence as is available. The author offers no such opening statement, although relevant comments are scattered through the book. He considers each major vascular group in turn, seeking an exact harmony between certain arbitrarily chosen xylem features and the habitat of the plant. The whole account is riddled with unsupported statements, special pleading and illogical handling of facts and ideas. The author never deals adequately with the phenotypic as opposed to genotypic differences between plants; slower-growing individuals generally have shorter, narrower xylem elements, but is this feature necessarily adaptive? There are some suggestive correlations of habitat with xylem cell form, but in respect of most of the detailed differences in xylem type one is bound to agree that "the logical conclusion to be drawn is that a variety of peculiar types are functionally quite effective" (p23). This book should have been condensed into a review article.

P. J. Grubb

Nuclear Quadrupole Resonance in Chemistry. By G. K. Semin, T. A. Babushkima and G. C. Yakobson. Pp. X+517. (Halsted, Wiley: New York and London; Israel Program for Scientific Translations: Jerusalem, October 1975.) £24.75.

THIS is a reasonably quick translation of an original published in the Russian in 1972. It does, however, have an inordinate price, considering that it has not even been set in normal print, but rather is the product of an electric typewriter. It is greatly to the credit of the authors that notwithstanding these shortcomings one is able to recommend it unreservedly to researchers in nuclear quadrupole resonance (NQR) spectroscopy, who will find 300 pages of data compilation invaluable (particularly because they contain so much from the Russian literature). The greatest emphasis is on the application of the technique to chemical problems and although the expert is so well catered for the authors have provided an adequate introduction to the technique and to the basic features of its theory. Any chemist reading the chapters which precede the data tables would acquire a sound feeling for the possible applications of NQR in his own field of research. What is not dealt with in detail, deliberately, is the quantum-mechanical calculation of coupling constants but this is no real shortcoming in a book which describes the chemical applications uniquely well.

The outstanding Russian contribution to this field of research is presented with modesty in a fascinating book.

K. A. McLaughlan

The Living Ocean: Marine Microbiology. (Biology and Environment.) By E. J. Ferguson-Wood. Pp. 146. (Croom Helm: London, September 1975.) £5.50.

I ENJOYED reading this book which is about the structure, ecology and economics of marine prokaryotes and protists. It was intended as a personal and selective account and has been rescued from an early draft prepared shortly before the author's death. It is therefore obviously open to some criticisms of approach and incompleteness. Ferguson-Wood's enthusiasm and involvement, however, are evident from the text which may be of most use as an infectious introduction to the subject. The book can be recommended to the beginner or layman, but cannot be regarded as an alternative to other accounts of microbiology, including the author's previous books, for more serious

students. Rather few references are given (in the guise of notes at the end of each chapter), some of these are rather trivial and more obvious important ones are omitted. The short title given on the cover and binding does not fairly indicate the book's content otherwise it is quite well produced with few minor errors —although some of the diagrams are poorly prepared. Professor Ferguson-Wood certainly contributed to the main course of marine microbiology; this book is a rather expensive aperitif.

P. J. S. Boaden

Books brief

Physiology of Physical Stress: A Selective Bibliography, 1500–1964. By Carleton B. Chapman and Elinor C. Reinmiller. Pp. vii+369. (Harvard University: Cambridge, Massachusetts and London, 1975.) £8.25.

THE compilers of this bibliography have selected 2,800 references concerned with some aspect of the physiology of physical stress. Physical stress seems to mean physical exercise or physical activity, to judge from the preface. References dealing with 'basic physiological work on physical stress' are included but 'extensions were made into clinical areas that had basic implications'.

The word 'stress' is notoriously difficult to define, and for that reason is best avoided whenever possible. The title might well have been 'The physiology of physical exercise'. It is difficult to determine why the authors include particular papers but not others. The word 'basic' is used five times in the third paragraph of the preface where an account is given of the identification of 'basic' articles. No doubt there would be considerable individual differences in the interpretation of 'basic' and thus decisions to include or reject must be to some extent arbitrary. All the same, I was surprised at the omission of Marius Nielsen's famous paper on the regulation of body temperature during physical exercise, and no reference is made to Adolph and *Physiology of Man in the Desert*. There are some other equally surprising gaps and some curious inclusions.

Nevertheless, one must applaud the determination required to prepare a bibliography which, in spite of some reservations, I have found useful.

O. G. Edholm

Electrode Kinetics. (Oxford Chemistry Series.) By John Albery. Pp. xii+184. (Clarendon: Oxford; Oxford University: London, June 1975.) £5.00.

ELECTRODE kinetics is a difficult subject, especially for the beginner, and so a new book setting down the fundamental ideas in a clear, lively and occasionally humorous manner is welcome. Many will also see this particular book as an original contribution to the subject. It is suitable for specialist first degree courses in electrode kinetics but especially for new research workers in the field. There is also much to interest the expert, with many new angles on old problems. The subject is presented in a way which should be intelligible to the beginner. Even so, the amount of detail is considerable and many of the finer points are discussed. Inevitably there is much mathematics, but the reader is helped through this by numerous sketches of the important functions. The choice of much of the material reflects the author's own interests. There is an excellent chapter on the double layer and an illuminating one on the theory of electron transfer which should interest a wider audience.

D. R. Whitehouse

Enzyme Induction. (Basic Life Sciences, Vol. 6.) Edited by D. V. Parke. Pp. xii+328. (Plenum: London and New York, 1975.) \$32.50. THE title of this interesting book, which covers a Colloquium held at the University of Surrey in June 1972, is rather deceiving. "Enzyme induction" has been coined for a phenomenon involving *de novo* synthesis of a specific protein in response to a specific signal in the environment, such as the synthesis of β -galactosidase or tryptophanase by *Escherichia coli*, in response to the presence of a β -galactoside or tryptophan in the growth medium.

Except the first chapter dealing exactly with phenomena of this type, the rest of the book deals mainly with the effects of drugs and steroids on the appearance of several enzymes at the cellular or developmental level. This appearance is not necessarily a reflection of a *de novo* synthesis, but may be the result of a decreased degradation attributable or not to a stabilisation of the enzyme studied.

The various contributions of the book will prove very useful to those interested in the mechanisms of biological regulation and their possible roles in health, ageing and disease. The chapter on induction of drug-metabolising enzymes present in liver endoplasmic reticulum is of special interest.

Georges N. Cohen

obituary

James Gray was born in London on October 14, 1891. His parents were both Scottish and he always felt himself to be an expatriate Scot. After early education at the Merchant Taylor's School he became first a Scholar and later a Fellow of King's College, Cambridge. Shortly after his election, his academic career was interrupted by the First World War, in which he served, with great distinction, as a Captain in the Queen's Royal West Surrey Regiment. He was awarded the Military Cross and had conferred upon him, by Marshall Foch in person on the field of battle, the Croix de Guerre with palms. After the war he returned to Cambridge to resume his Fellowship and soon to be married, in 1920, to Norah Christine King, who survives him. Not long after this he was appointed Lecturer, later Reader, and in 1937 he was elected to the Chair of Zoology which he occupied until he reached the retiring age, in 1959. During retirement he continued to reside in Cambridge, where he died peacefully in his own home on December 14, 1975.

Gray's first research was concerned with the fertilisation and early development of echinoderm eggs, but already he was a cell biologist rather than an embryologist. It was primarily his interest in cells that led him on to the study of ciliary movement on which he quickly became the world authority, as was recognised by his election to Fellowship of the Royal Society in 1929. After writing a notable book, *Experimental Cytology*, he made up his mind to leave this field; he felt that, using living cells, means were not then available to formulate meaningful questions and to obtain clear-cut answers—and he was not interested in dead cells. In deciding to move into the field of locomotion he was no doubt influenced by the circumstance that he had developed the techniques of cinematography for use on cilia, and these techniques could be applied to other types of movement. Animal locomotion occupied him over the next 25 years, bringing him the award of the Royal Medal of the Royal Society in 1948, and it is for his work in this field that he will be long remembered. His calculation that the power required to drag a dolphin's dead body through water is greater than the power available from its muscles has become

widely known as Gray's Paradox. From the swimming of dolphins to the swimming of spermatozoa, from the movement of snakes to the movement of minute nematode worms he explored the undulatory mode of propulsion, and also investigated, in collaboration with H. W. Lissmann, the modes of terrestrial locomotion seen in amphibia. During the early part of his retirement he was able to bring all this work together in his second great book, *Animal Locomotion*.

Later in life Gray became increasingly involved in advisory work for national organisations. He was a trustee of the British Museum (1948–60), a member of the Agricultural Research Council (1942–47), President of the Marine Biological Association (1945–55) and President of the British Association in 1959. From 1951–59 he was a member of the Development Commission and at one time Chairman of its Fishery Advisory Committee. For his public work he was made CBE in 1946, and in 1954 he received a Knighthood. He was honoured by many universities, by Aberdeen and Edinburgh with the L.I.D., by Durham, Manchester and Wales with the D.Sc.

Gray's influence upon the development of zoology was greater even than his direct contribution to it. He was foremost among a band of rising young biologists who in the years immediately after the First World War led the subject into new paths and established Experimental Zoology in the face of traditionalist opposition. He continued to foster this movement through the Society for Experimental Biology, at whose meetings he was regularly to be seen, and through the *Journal of Experimental Biology* which he edited from 1925 to 1954. His influence was, naturally, nowhere stronger than in his own university. During his tenure of the Chair the teaching of Zoology was transformed—but gradually, for he was emphatically opposed to unnecessary disturbance and dislocation. This was a time when the director of research and his team of research students were beginning to make their appearance on the university scene. Gray would have none of this. He looked upon research students as independent research workers in their own right; as long as they were swimming he left them alone, if ever they got into serious

difficulty they were dramatically rescued. Gray believed passionately that the mainspring of a successful university department was the activity of the staff in research. What line of research they chose mattered nothing provided that it was scientifically sound and pursued with enthusiasm; for, he argued, this enthusiasm would rub off on the undergraduates and, far more than mere knowledge imparted to them, would bring out their best qualities. One has but to look at the list of zoologists who were at one time research students in Gray's department and then went off to become Heads of Departments themselves to see how well the system worked.

As Head of Department himself he was always approachable, always ready to listen to someone in trouble, always ready to step in where he could be of help. There was also a rough side to his character. He was slow to wrath, but it was always a grave matter when he was aroused. Yet he was scrupulous to keep his personal feelings to himself, and they were never allowed to influence his official policy or decisions. In private life, though he had many very close friends, his happiest hours were spent on summer holidays in Scotland with his family.

J. A. Ramsay

Joseph F. Foster, Professor of Chemistry in Purdue University and former Chairman of the Department, died suddenly on October 7, 1975 at the age of 57. He was distinguished for his research on the physical chemistry of macromolecules, especially on starch and proteins. He was probably the foremost contributor to our knowledge of the serum albumin molecule during the last twenty years. He demonstrated its reversible expansion in acid solutions, and discovered two Conformational transitions in albumin, one in acid solution (the $N \rightleftharpoons F$ transition) and another in neutral slightly alkaline solution. From this he inferred and demonstrated the microheterogeneity of albumin preparations. He was extending and deepening these important studies up to his untimely death. He will be greatly missed by his colleagues in protein chemistry.

John T. Edsall

announcements

Awards

In addition to the winners already mentioned of the prizes in Cancer Immunology awarded by the Cancer Institute of New York, were also: **Dr Garri Abelev**, N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow, and **Dr Eva Klein**, Karolinska Institute, Stockholm, Sweden.

Appointments

Dr Michael Gibbons has been appointed Professor of Liberal Studies in Science at the University of Manchester.

Dr W. H. Cockcroft, at present the G. F. Grant Professor of Pure Mathematics at the University of Hull, is to become Vice-Chancellor of the New University of Ulster in October.

International meetings

March 1-3, **First World Hydrogen Energy Conference**, Miami Beach, Florida (1st World Hydrogen Energy Conference, Conference Services, University of Miami, PO Box 248005, Coral Gables, Florida 33124).

March 15-19, **Development of Nuclear-based Techniques for the Measurement, Detection and Control of Environmental Pollutants**, Vienna (The IAEA Kärntner Ring 11, PO Box 590, A-1011 Vienna).

March 22-26, **The Management of Radioactive Wastes**, Vienna (The IAEA Kärntner Ring 11, PO Box 590, A-1011 Vienna).

March 29-April 2, **The Exploration of Uranium Ore Deposits**, Mexico City (The IAEA, Kärntner Ring 11, PO Box 590, A-1011 Vienna).

Miscellaneous

The **Lady Tata Memorial Trust** offers fellowships and scholarships for research on leukemia and allied conditions. The fellowships are tenable for 3 years and the scholarships for 1 year, renewable for 2 more. For further particulars apply to The Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Institute, Fulham Road, London SW3. Applications must be submitted by March 31.

Reports and publications

Smithsonian Contributions to Zoology, No. 204: Taxonomic Indexes to Ostracoda (Suborder Myodocopa) in Skogsborg (1920) and Poulsen (1962, 1965).

Person to Person

The change in the tensile strength of cellulose on burial in soil is a technique of general application in ecology, but for repeatable results, a high-quality standard test cloth must be available. The Shirley Institute, Manchester has agreed to manufacture, and sell at cost price, such a material if initial demand is sufficient. Anyone who might be interested should contact Dr D. W. H. Walton, British Antarctic Survey, Monks Wood Experimental Station, Abbots Ripton, Huntingdon, Cambs. PE17 2LS.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

By Anne C. Dohen and Louis S. Kornicker. Pp. iii + 29. No. 209: Vertical Distribution of Pkagic Cephalopods. By Clyde F. E. Roper and Richard E. Young. Pp. iv + 51. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.)

[1811] United States Department of the Interior: Geological Survey. Bulletin 1389: Stratigraphic and Structural Relationships of the Brimfield Group in Northeast-Central Connecticut and Adjacent Massachusetts. By John D. Peper, M. H. Pease, Jr., and Victor M. Seiders. Pp. iv + 31 + 3 plates. Water-Supply Paper 2101: Surface Water Supply of the United States 1966-70. Part 1: North Atlantic Slope Basins. Vol. 1: Basins from Maine to Connecticut. Pp. xi + 123. Water-Supply Paper 2143: Quality of Surface Waters of the United States, 1969. Part 3: Ohio River Basin. Pp. x + 371. \$2.85. Professional Paper 437-E: Land Subsidence Due to Ground-Water Withdrawal in the Los Banos-Kettleman City Area, California. Part 1: Changes in the Hydrologic Environment Conductive to Subsidence. By William B. Bull and Raymond E. Miller. Pp. iv + 71. Professional Paper 725-B: Generalized Geologic Framework of the National Reactor Testing Station, Idaho. By Raymond L. Nace, Paul T. Voegell, James R. Jones, and Morris Deutsch. Edited by Seymour Subitzky. Pp. iv + 49 + plate 1. Professional Paper 798-B: Geology and Geochemistry of the Copper Canyon Porphyry Copper Deposit and Surrounding Area, Lander County, Nevada. By Ted G. Theodore and David W. Blake. Pp. v + 86 + 2 plates. (Washington, DC: Government Printing Office, 1975.)

[1811] United States Department of the Interior: Geological Survey. Bulletin 1395-B: Revision of the Type Mankom Formation (Pennsylvanian and Permian), Eagle Creek Area, Eastern Waska Range, Alaska. By D. H. Richter and J. T. Dutro, Jr. Pp. iii + 25. Water-Supply Paper 2030: Ground-Water Levels in the United States, 1967-71. North-Central States. Pp. v + 114. 80 cents. (Washington, DC: Government Printing Office, 1973 and 1975.)

[1911] Annals of the South African Museum. Vol. 67, Part 6: Pleistocene Molluscs from the West and South Coasts of the Cape Province, South Africa. By R. N. Kilburn and A. J. Tankard. Pp. 183-226. R3.55. Vol. 67, Part 7: The Morphology and Relationships of a Crocodilian, *Orthosuchus stormbergi*, from the Upper Triassic of Lesotho. By Diane S. Nash. Pp. 227-329. R6.30. Vol. 67, Part 8: Archaeocyatha Provenant de Blocs Erratiques des Tillites de Dwyka (Afrique du Sud). Par Francoise Debrenne. Pp. 331-361. R2.90. Vol. 67, Part 9: A New Species of *Meiosquilla* (Crustacea, Stomatopoda) from South Africa. By Raymond B. Manning. Pp. 363-366. R1.50. Vol. 67, Part 10: Five Species of *Jaeropsis* from the Southern Indian Ocean

(Crustacea, Isopoda, Asellota). By Brian Kensley. Pp. 367-380. R1.85. Vol. 67, Part 11: A Preliminary Catalogue of Identifiable Fossil Fish Material from Southern Africa. By R. A. Jubb and B. G. Gardiner. Pp. 381-440. R4.40. (Cape Town: South African Museum, 1975.)

[2011] World Health Organisation. Technical Report Series No. 577: Evaluation of Dependence Liability and Dependence Potential of Drugs—Report of a WHO Scientific Group. Pp. 50. (Geneva: WHO; London: HMSO, 1975.) Sw. fr. 7.

[2111] Smithsonian Contributions to Zoology. No. 191: Cyclopoid Copepods (Lichomolgidae) Associated with Alcyonaceans in New Caledonia. By Arthur G. Humes. Pp. iii + 27. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.)

[2111] World Health Organisation. Technical Report Series. No. 575: Advances in Methods of Fertility Regulation—Report of a WHO Scientific Group. Pp. 45. (Geneva: WHO; London: HMSO, 1975.) Sw. fr. 6.

[2411] United States Department of the Interior: Geological Survey. Professional Paper 433-0: Distribution of Radionuclides in the Columbia River Streambed, Hanford Reservation to Longview, Washington. By W. L. Haushild, G. R. Dempster, Jr., and H. H. Stevens, Jr. Pp. iv + 35. Professional Paper 502-C: Solute Balance at Abert and Summer Lakes, South-Central Oregon. By A. S. Van Denburgh. Pp. iv + 29 + 1 plate. Professional Paper 813-B: Summary Appraisals of the Nation's Ground-Water Resources—Upper Mississippi Region. By R. M. Bloyd, Jr. Pp. iii + 22. Professional Paper 836: Stratigraphic Distribution and Zonation of Jurassic (Callovian) Ammonites in Southern Alaska. By Ralph W. Imray. Pp. iii + 28 + 6 plates. Professional Paper 864-A: A Review and Interpretation of the Geologic Setting of the Watchung Basalt Flows, New Jersey. By George T. Faust. Pp. iii + 42. (Washington, DC: Government Printing Office, 1975.)

[2411] World Health Organisation. Technical Report Series. No. 576: Evaluation of Certain Food Additives—Some Food Colours, Thickening Agents, Smoke Condensates, and Certain Other Substances. (Nineteenth Report of the Joint FAO/WHO Expert Committee on Food Additives.) Pp. 23. Sw. fr. 5. No. 579: Developments in Malaria Immunology—Report of a WHO Scientific Group. Pp. 68. Sw. fr. 7. (Geneva: WHO; London: HMSO, 1975.)

[2711] United States Department of the Interior: Geological Survey. Bulletin 1382-C: Argentinian Cryptomelane and Bromargyrite in Volcanic Rocks near Silver Cliff, Colorado. By Fred A. Hildebrand and Elwin L. Mosier. Pp. iii + 23. (Washington, DC: Government Printing Office, 1974.) 40 cents.

[2711] United States Department of the Interior: Geological Survey. Water-Supply Paper 2007—Geologic and Hydrologic Features of Indian Wells Valley, California. By L. C. Dutcher and W. R. Moyle, Jr. Pp. iv + 30 + 6 plates. (Washington, DC: US Government Printing Office, 1973.) \$3.75.

[2811] Nederlandse Vereniging voor weer- en Sterrenkunde. Observations of Variable Stars, January/June 1975. (Report No. 28.) Pp. 10. (Groningen, Netherlands: Kapteyn Astronomical Laboratory, 1975.)

[112] OECD Halden Reactor Project—Fifteenth Annual Report. Pp. 103. (Paris: OECD, Nuclear Energy Agency, 1975.)

[112] Republic of Cyprus. Ministry of Agriculture and Natural Resources. Annual Report of the Geological Survey Department for the year 1974. By Y. Hadjiistavrinou. Pp. 45. (Nicosia: Geological Survey Department, 1975.)

[212] CERN: European Organization for Nuclear Research. CERN-75-13: AGS—The ISR Computer Program for Synchrotron Design, Orbit Analysis and Insertion Matching. By E. Keil, Y. Marti, B. W. Montague and A. Sudboe. Pp. vi + 57. (Geneva: CERN, 1975.)

[212] Académie Royale de Belgique. Mémoires de la Classe des Sciences, 2^e Série. T.XLI, Fasc. 7: La Théorie de la Variation Seconde des ses Applications en Commande Optimale. Par R. Henrion. Pp. 208. (Bruxelles: Académie Royale de Belgique, 1975.)

[212] Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel, Exercice 1974. Pp. 507. (Bruxelles: IRSIA, 1975.)

[212] CERN—European Organization for Nuclear Research. CERN 75-14: Monte Carlo Calculations of the Neutron Transmission Through the Access Ways of the CERN Super Proton Synchrotron. By H. G. Vogt. Pp. v + 37. (Geneva: CERN, 1975.)

[412] New Zealand: Department of Health. National Radiation Laboratory. Environmental Radioactivity—Annual Report for 1974. Pp. 38. (Christchurch, NZ: National Radiation Laboratory, 1975.)

[412] United States Department of the Interior: Geological Survey. Professional Paper 773: Geologic Structures in the Gulf of Mexico Basin. By Louis E. Garrison and Ray G. Marini, Jr. Pp. iv + 85 + 1 plate. (Washington, DC: US Government Printing Office, 1973.) \$1.75.